TUBERCULOSIS RESEARCH CENTRE

Research Activities

April 2007 – March 2008

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Distinguished Visitors

Date visited	Name	Organistaion/place	Remarks
10.05.07	Dr.Andrews Kostler	Tibotec	“Impressive scientific knowledge and equally impressive operational/logistic setting”
10.05.07	Dr.Tine De Mauz	Tibotec	“Impressed with long time TB experience and clinical trial experience and hoping for future collaborations”
10.05.07	Mr.Santhosh Shevade,GCO	Johnson and Johnson, India	“Looking forward to a continued interaction and continuing support”
27.07.07	Case Gordon	TB Activist, France	“Thank you very much”
27.07.07	Dr. Thomas Mampilly	National Institute of Health, USA	“Very impressive! Thank you for taking the time/effort to show me around”
25.01.08	Euken Baneva	Development Agency, Basque country	“Very impressive work. Wish you all success”
25.01.08	Igor Irigoyon	Director of Co-operation, Basque country	“Thank you for your warm welcome and above all for your work”
25.01.08	Olga Pedrova	Development Agency, Basque country	“Congratulations for the really good team work. Thanks for everything”
25.01.08	Ana Gartalot	Development Agency, Basque country	“Hoping that one day all TRC efforts and work will be compensated and we could celebrate that AIDS is a part of the past”

ABBREVIATIONS

3TC	Lamivudine
ACR	Allelic crystalline protein
AES	Allelic exchange substrate
AFB	Acid fast bacilli
ANN	Artificial neural network
ART	Antiretroviral therapy
ARTI	Acute respiratory tact infection
ARV	Antiretorviral drugs
ASOP	Allele specific oligonucleotide probes
ATT	Anti-TB treatment
AZT	Zidovudine
BCG	Bacillus Calmette Guerin
BL	Blood cells
BMI	Body mass index
CBA	Cytometric bead array
CFA	Culture filtrate antigen
CFP	Culture filtrate protein
CFU	Colony forming unit
CPC	Cetyl pyridinium chloride
CS	Chest symptomatic
CYP2B6	Cytochrome P450 2B6
DC	Dendritic cells
d4T	Stavudine
ddI	Didanosine
DOTS	Directly observed treatment short-course
DNA	Deoxyribonucleic acid
DR	Direct repeat
DRM	Drug resistance mutation
DST	Drug susceptibility testing
DSMB	Data safety monitoring board
EAI	East African Indian
ECG	Electrocardiogram
EDP	Electronic data processing
EFV	Efavirenz
ELISA	Enzyme linked immunosorbent assay
EMB	Ethambutol
EMSA	Electrophoretic mobility shift assay
Env	Envelope
ESAT	Early secreted antigenic target
EQA	External quality assurance
FDA-EB	Fluorescein diacetate-ethidium bromide
FDC	Fixed dose combinations
FGDs	Focus group discussions
GFATM	Global fund against AIDS, Tuberculosis and Malaria

GLAS	Graphical library automation system
GM-CSF	Granulocyte macrophage colony stimulating factor
HAART	Highly active antiretroviral therapy
HIV	Human immuno deficiency virus
HLA	Human Leucocyte Antigen
HPLC	High performance liquid chromatography
HHC	Healthy household contacts
HIVVT	HIV vaccine trial
HR	Heart rate
HRQoL	Health related quality of life
HSV	Herpes simplex virus
HTH	Helix turn helix
ICMR	Indian Council of Medical Research
IcIR	Isocitrate lyase regulatory protein
IID	Independent and identically distributed
IGRA	Interferon gamma releasing assay
INH	Isoniazid
IRL	Intermediate reference laboratory
LIA	Line immuno assay
LJ	Lowenstein-Jenson
LPS	Lipopolysaccharide
LRP	Luciferase reporter phage
O	Ofloxacin
ORFs	Open reading frames
MAPK	Mitogen activated protein kinases
MBL	Mannose binding lectin
MCMC	Markov chain Monte Carlo
MFX	Moxifloxacin
MDR	Multi drug resistance
MDR-TB	Multi-drug resistant tuberculosis
MIC	Minimal inhibitory concentration
MIRU-VNTR	Mycobacterial interspersed repeat unit-Variable number of tandem repeats
MLH	Mothers living with HIV-AIDS
MOI	Multiplicity of infection
MoDC	Monocyte derived DCs
MSM	Men having sex with men
MVA	Modified Vaccine Ankara
NACO	National AIDS Control Organization
NGO	Non-Governmental Organization
NK	Natural killer
NRL-OSE	National reference laboratory-onsite evaluation
NRTI	Nucleoside reverse transcriptase inhibitors
NNRTI	Non-NRTI
NVP	Nevirapine
PBMC	Peripheral blood mononuclear cells

PC	Personal computers
PCR-ASP	Polymerase chain reaction – allele specific primer
PCR-RFLP	Polymerase chain reaction – Restriction fragment length polymorphism
PCR-SSP	Polymerase chain reaction – sequence specific primer
PDF	Portable document file
PF	Pleural fluid
PFMC	Pleural fluid mononuclear cells
PLAHs	People living with HIV-AIDS
PHC	Primary health centre
PMA	Phorbal myristate acetate
PPs	Private practitioners
PPD	Purified protein derivative
Prot	Protease
PZA	Pyrazinamide
PZAase	Pyrazinamidase
QFT-G	Quantiferon TB gold kit
RBRC	Random blinded checking
RMP	Rifampicin
RNTCP	Revised National TB Control Programe
RT	Reverse transcriptase
SCC	Short-course chemotherapy
SDS	Sodium dodecyl sulphate
SLC	Secondary lymphoid chemokine
SRP	Signal recognition particle
SSIG	Semi-structured interview guide
STDC	State TB demonstration centre
STPK	Serine/threonine protein kinases
TB	Tuberculosis
TEM	Transmission electron microscopy
TLR	Toll like receptors
TMP	Tape measure protein
TP	Tuberculous pleuritis
TR	Transcriptional regulators
TSS	Transcriptional start site
TU	Tuberculosis unit
USAID	United States Agency for International Development
VDR	Vitamin D receptor
VNTR	Variable number tandem repeat
WHO	World Health Organization
XDR-TB	Extensively drug-resistant TB

CLINICAL RESEARCH

Completed studies

Evaluation of chemotherapy regimens for tuberculosis in HIV-infected persons

(Funded by ICMR Task Force on HIV-TB)

Background

The duration of anti-tuberculosis (TB) treatment among Human Immuno Deficiency Virus (HIV)-positive patients with TB is still a contentious issue. A 6-month intermittent (3 times/week) regimen is the standard treatment for TB in the Revised National Tuberculosis Control Programme (RNTCP) in India and many countries.

Aims

- To evaluate the efficacy of RNTCP Category-I treatment regimen ($2EHRZ_3/4RH_3$) among HIV positive patients infected with TB and compare it with a 6-month versus a 9-month intermittent anti-TB regimen with respect to reduction in failure and relapses

Methods

This was a randomized, controlled clinical trial with two arms, a 6-month regimen $2EHRZ_3/4RH_3$ and a 9-month regimen $2EHRZ_3/7RH_3$ E: (ethambutol) 1200 mg, H: (isoniazid) 600 mg, R: (rifampicin) 450mg in patients with <60kg/600 mg > 60 kg and Z: (pyrazinamide) 1500 mg with pyridoxine 10 mg, given thrice weekly throughout.

All HIV-positive patients diagnosed with pulmonary or extrapulmonary TB based on sputum smear and culture, fine needle aspiration cytology/biopsy/biochemical investigations of pleural fluid were included in the study if they fulfilled other eligibility criteria. Randomization was done in permuted block of four and stratified by CD4 cell count (<200 & >200 cells/mm^3), and smear grading (0, 1+, 2+ and 3+). Treatment was fully supervised for the first 2 months, followed by once a week. In cases of sputum smears being positive at the end of 2^{nd} month, the intensive phase was extended by 4 weeks. Patients were followed up every month with clinical examination, sputum acid fast bacilli (AFB) smear and culture

for *M. tuberculosis*. Chest radiograph and CD4 counts were performed at baseline, 2nd month and at the end of therapy. None of the patients were on antiretroviral therapy (ART) during the treatment period. End points of the study were sputum culture negativity at the end of treatment and relapses up to 36 months follow-up (clinical and/or bacteriological). Intent to treat and on-treatment analysis was performed.

Results

Study population: Three hundred and thirty four patients were admitted and randomized to the study regimens. There were 7 exclusions in the efficacy analysis (4 - primary multi-drug resistant (MDR), 2 had regimen changed due to need of ART containing nevirapine and 1 patient had culture positive for *M.kansasii*). Out of the remaining 327 cases, 167 were allocated to 6-month regimen and 160 to the 9-month regimen. Two hundred and twenty seven of these patients had sputum culture confirmed TB: 117 were allocated to 6-month regimen and 103 to 9-month regimen. Seventy three cases were culture negative for pulmonary TB with clinico-radiological features including miliary and mediastinal TB and 27 cases had extrapulmonary TB

The baseline characteristics of study population are given in table 1.

Table 1: Baseline characteristics

	Regimen 6M N = 167	Regimen 9M N = 160
Males (%)	78	74
Mean Age (Years) +range	33.7 (17 - 60)	34.3 (18 - 63)
Mean Weight (kg)+ range	44.4 (27.6 - 65)	44.3 (18.5 - 73.7)
Sputum smear (% positive)	54	54
Sputum culture (% positive)	69	68
Median CD4 (cells/cu.mm) (inter-quartile range)	152 (82 – 304)	167 (83 – 270)

Outcome: At the end of treatment, 138 (83%) in 6-month regimen and 122 (77%) in 9-month regimen had a favourable response; the difference was non

significant. There were 8 & 11 bacteriological failures and 4 & 5 patients with clinical deterioration in regimens A and B respectively. One case in regimen A and 3 cases in regimen B died due to TB during treatment. The regimen was changed due to toxicity to ATT in one patient in each of the regimens, and one patient in 9-month regimen required nevirapine containing ART along with change of ATT.

The toxicity profile showed that 22 % of patients had minor toxicity, 3 cases had ATT temporarily withheld due to jaundice which was successfully reintroduced. Only two patients had a permanent change in the regimen due to cutaneous toxicity to ATT. Drug toxicity was similar in both the groups.

Baseline characteristics and outcome in sputum culture positive pulmonary TB: Out of the 212 sputum culture confirmed pulmonary TB in the efficacy analysis (received > 80% drug doses), 54% had smear positivity at start of treatment. Two-thirds of the patients had severe immunosuppression as evidenced by CD4 counts < 200 cells/mm^3. 89% of the patients had pretreatment culture with *M. tuberculosis* sensitive to all first line anti-TB drugs. At the end of intensive phase, culture conversion was observed in 87% of the patients. Outcome at the end of treatment is given in table 2.

Table 2: Outcome in sputum culture positive patients

	6-month regimen (N=109)	9-month regimen (N=103)
Favourable response	93 (85%)	80 (78%)
Unfavourable responses		
	16	23
Bacteriolgoical failure	8	11
Clinical deterioration	3	1
TB death	1	3
Non-TB death	3	8
Others	1	0

In patients with culture positive TB and declared cured, 19 (20%) in 6-month regimen and 7 (9%) in 9-month regimen had a bacteriological recurrence within 36 months from the time of starting treatment ($p < 0.05$). Deaths durind follow-up were similar in both the groups. The study results suggest that even though the 6-month and 9-month anti-TB regimens had similar efficacy at the end of treatment, there was a significant reduction in bacteriological recurrences with longer regimen of ATT. However, the 9-month regimen did not improve survival at 36 months, in the setting of limited access to ART.

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

Preventive therapy for TB among HIV-infected individuals

(Funded by United States Agency for International Development)

Background

Available evidence indicates that preventive therapy for TB reduces the frequency of active TB in HIV positive subjects by about 50% to 60%. Protection is greatest in adults with a positive tuberculin skin testing (70% reduction in incidence, mortality reduced 25%). However, the ideal duration of preventive therapy especially in TB-endemic countries is not known.

Aim

- To study the efficacy of two different preventive therapy regimens [6 months of ethambutol (EMB) & isoniazid (INH) and 3 years of INH] in HIV-infected persons in reducing the incidence of TB and overall mortality

Outcome measures

- Development of pulmonary or extra-pulmonary TB
- Death due to TB

Study design

The study was conducted as a two-armed randomized clinical trial among HIV-positive patients without active TB.

The treatment regimens were as follows:

- EMB (800 mg) and INH (300 mg) daily for six months, self-administered, collected once in 15 days

- INH (300 mg) daily for 3 years (in lieu of lifelong prophylaxis) self-administered, collected once in 15 days

Subjects in both study groups received 10 mg of pyridoxine daily during treatment.

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 every three months. Patients suspected to have TB at any time were completely investigated and treated appropriately. Any positive culture was subjected to drug susceptibility tests. Attempts were made to ascertain the cause in all cases of death.

Results

Of the 712 patients admitted to the study from March 2001 – September 2005, 632 were eligible for analysis. The baseline characteristics of the study patients are shown in table 3. The mean age, weight, CD4 cell count and mantoux test reaction were comparable in both the groups.

A total of 247 patients from the INH arm and 283 from the EMB/INH arm have completed 36 months of follow-up as of 31st March 2008.

Twenty patients in the EMB/INH arm and 15 in the INH group developed active TB giving a breakdown rate of 1.76 / 100 person years. Most of the breakdown in both the arms occurred in the first 12 months. There were 30 deaths in the EMB/INH arm while 23 deaths had occurred in patients who were on INH.

The toxicity pattern in both the groups was similar. Only one patient in the INH arm had termination of treatment because of severe jaundice.

The interim findings suggest that the 6 months of EMB/INH regimen is as effective as 3 years of INH in preventing TB among HIV-infected persons. Patients with lower CD4 cell count were at higher risk of TB breakdown and death.

Table 3: Baseline characteristics

	EMB/INH – 6 Months		INH- 36 Months	
	n = 318		n = 314	
	Mean±S.D	Range	Mean±S.D	Range
Age (yrs)	29 ± 7	18 -57	30 ± 6	18 - 30
Wt (kgs)	51± 10	32 - 79	50 ± 11	30 - 97
CD4 (cells/mm^3	337±257	35-1125	330±224	12-1247
Mx (mm)	9 ± 10	0.0-40.0	8 ± 9	0.0-35.0

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

A clinical trial to study the efficacy of two different once-daily antiretroviral regimens along with anti-TB treatment in patients with HIV-1 and TB
(Funded by National AIDS Control Organization)

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 in HIV-infected TB patients. The specific aim was to study a once-daily regimen of didanosine + lamivudine (3TC) + efavirenz (EFV) or nevirapine (NVP) along with standard ATT in patients with HIV and TB with CD4 cell counts < 250 cells/mm^3. The primary outcome measure was suppression of viral load (< 400 copies/ml) after 24 weeks of ART. A secondary outcome was to study the utility of DOT in this setting vs. self-administered ART. Pharmacokinetic studies were also planned concurrently to study the interaction between rifampicin (RMP) and NVP/EFV as well as monitoring for development of drug resistance.

The pilot study was initiated in October 2005 with recruitment at Chennai, Madurai and Vellore. 57 patients (43 males and 14 females) were screened, of whom 20 were recruited to the study. These 20 patients consisted of 17 males and 3 females, their age ranging between 22–44 years. Following the pilot study

which lasted for one year, 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 March 31, 2008, 564 patients had been screened for the study at 5 centres (including 3 sites in Chennai, 1 in Vellore and 1 in Madurai). 130 patients were admitted to the study (patient demographics in table 4). All patients were randomized at the end of intensive phase (2 months) of anti-TB treatment: 70 patients received the EFV regimen and 57 the NVP regimen, 3 patients died before randomization. 79 of these patients had pulmonary TB while the rest had extrapulmonary TB (pleural effusion, TB lymph node). Response to anti-TB treatment was good with 87.5% of patients becoming culture negative at 2nd month and 96% of those who completed 6 months culture negative. Overall, cure/completion rate was approximately 85%. Only one patient had an adverse reaction to anti-TB drug which required a change of treatment.

At the end of 6 months of ART, of the 70 patients randomized to receive EFV, 14 are still on treatment, 47 have completed with viral load <400 copies/ml, 5 had virologic failures, one patient died and one defaulted. In the NVP regimen, 37 of 57 have completed with viral load < 400 copies/ml, 9 had virologic failure, 5 died, 3 defaulted and 2 are still on treatment. There have been 8 serious adverse events equally distributed in both the 2 arms.

Overall, the response to treatment has been satisfactory with good improvement in CD4 cell counts (Table 5).

The DSMB met on December 15, 2007 (for first interim analysis) and recommended that intake to the NVP withheld till further analysis. This was in view of the higher failure rates and deaths in the NVP arm compared to the EFV arm.

The 2nd interim analysis was done and presented to the DSMB on June 14, 2008. The DSMB recommended that intake to the study can be stopped as the primary question had been answered.

Table 4: Patient demographics

	Efavirenz (n= 70)	Nevirapine (n= 57)
Sex (M, F)	55, 15	44, 13
Age (years)	35 ± 7.5	38 ± 7.8
Height (cm)	161 ± 8.4	159 ± 7.1
Weight (kg)	42.7 ± 8.5	41.6 ± 7.3
Median CD4 (cells/mm^3) IQR	85 47-85	83 33-134.5
Viral load (copies /ml) Median (range)	3,62,000 (41575 - 7,50,000)	2,82,000 (1,28,500 – 6,49,500)
BMI	16.3 ± 2.7 (9.4 – 23.8)	16.4 ± 2.4 (10.8 – 22.4)

Table 5: Change of CD4 with ART

	Baseline	0 week	1 month	4 month	6 month
Efavirenz	95 ± 58	125 ± 84	255 ± 141	275 ± 125	325 ± 173
Nevirapine	87 ± 60	132 ± 82	238 ± 163	247 ± 166	283 ± 169

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

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 is aimed to identify other immunologic 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 Organization (NACO) guidelines.

Aims

- To investigate the relationship of naïve CD4 T-cells with total CD4 T-cells at study entry and prospectively over the course of the disease with and without ART
- To determine the relationship of $CD8^+$ T-cells expressing CD127 (IL7Rα), the receptor for cytokine IL-7 with disease progression
- To determine the relationship of dendritic cells (numbers and function) with immunologic status

Methods

Sixty ART naïve HIV-positive children (28 males/32 females) were screened and recruited to the study. Age ranged from 9 months to 13 years with median body mass index (BMI) of 14.5 (6.4–24.1). CD4 counts in the study subjects ranged between 131–2396 (median=728) cells/mm^3 and CD8 counts between 573–6086 (median=1585) cells/mm^3. At each visit, all children were examined clinically and venous blood samples were collected for immunological and other assessment as per the following schedule:

Schedule of evaluation

	Weeks				
	0	12	24	36	48
Clinical	✓	✓	✓	✓	✓
CBC / diff	✓	✓	✓	✓	✓
Routine Biochemical Test	✓		✓		✓
HIV RNA	✓		✓		✓
CD4 / CD8	✓	✓	✓	✓	✓
*Special Immunology	✓	✓	✓	✓	✓
* Naïve CD4, TREC, IL7Rα in CD8 T cells, DR^+CD38^+ CD8 T cells, DC phenotype and function					

The children will be followed up to 48 weeks, after which they will be referred to the nearest ART centre for management.

Results

A summary of the patients enrolled in the study and their follow-up as of May 2008 are given in table 6.

Table 6: Details of patient enrollment

	Baseline	1st follow-up (12 Weeks)	2nd follow-up (24 Weeks)	3rd follow-up (36 Weeks)	4th follow-up (48 Weeks)
No. of Children	60	56	48	36	25

Nine children had been started on ART while 51 were not; all were monitored regularly as per treatment guidelines and protocol.

The major findings of the study were that naïve ($CD45RA^{+}CD62L^{+}$) cells were greater in CD4 T- cells as compared to CD8 T-cells; in both T-cell subsets (CD4 and CD8) they directly correlated with CD4%. Significant increase in the naïve CD4 T cells was observed in all the patients who were on ART. Expression of immune activation markers ($CD38^{+}HLA^{-}DR^{+}$) was greater in CD8 T-cells and they inversely correlated with CD4% in both subsets (CD4 and CD8). A decrease in the immune activation markers was observed in all the patients who were on ART.

Expression of CD127, (IL-7Rα), a marker for memory cells, was reduced mainly on CD8 T-cells and its expression correlated directly with CD4%. Although, the expression of CD127 on $CD4^{+}$ T cells was low, it was not significant. Significant increase in the expression of CD127 was observed in ART treated individuals.

Reduced number of DC subsets (mDC and pDC) was observed in HIV-infected children who were not on ART. We observed significantly greater expression of CD80 and CD83 in unstimulated versus resiquimod stimulated whole blood. Their pDC subsets were responsive to stimulation by the TLR7/8 agonist Resiquimod to produce IFN-γ and TNF-α. Interestingly, pDC TNF-α production

was highly significant and inversely correlated to immune activation status in children with normal CD4 counts.

Conclusion

In summary, immunologic assessments indicate that loss of naïve CD4/CD8 T-cells and $CD127^{+}$ CD8 T-cells are associated with immune activation along with decrease in CD4 T-cells. This may prove useful as markers of disease severity or recovery in HIV-infected children initiating ART. pDC functional deterioration was not apparent in relation to CD4 counts, but did correlate inversely to immune activation.

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

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 TB

(PROVCTRI/2008/091/000024)

Background

Earlier clinical trials by the Tuberculosis Resarch Centre (TRC) have shown that while a 4-month daily regimen which included ofloxacin in the intensive phase was successful in the treatment of sputum positive pulmonary TB, 4-month thrice weekly regimens with ofloxacin, gatifloxacin or moxifloxacin (MFX) were less successful with high relapse rates. Following publication of the TRC clinical trial demonstrating the efficacy of an ofloxacin containing regimen in shortening TB treatment to 4 months (Indian Journal of Tuberculosis 2002), there has been a global interest in the role of the quinolones in the primary treatment of TB. MFX is now considered the most effective of the quinolones for treatment of TB due to its many unique qualities. The TRC is now conducting a 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 TB. Newly diagnosed smear positive pulmonary TB patients are randomly allocated to 3-month or 4-month MFX regimens or a control 6-month regimen. The regimens being tested are as follows:

Regimens

3 RHZEM (RMP, INH, PZA, EMB and MFX daily for three months (treatment duration 3 months);

2 RHZEM/ 2 RHM (RMP, INH, PZA, EMB and MFX daily for two months followed by RMP, INH and MFX daily for two months (treatment duration 4 months);

2 RHZEM/ 2 RHM thrice weekly (RMP, INH, PZA, EMB and MFX daily for two months followed by RMP, INH and MFX thrice weekly for two months (treatment duration 4 months);

2 RHZEM/ 2 RHEM thrice weekly (RMP, INH, PZA, EMB and MFX daily for two months followed by RMP, INH, EMB and MFX thrice weekly for two months (treatment duration 4 months)

2 RHZE thrice weekly/ 4 RH thrice weekly (RMP, INH, PZA and EMB thrice weekly for two months followed by RMP and INH thrice weekly for four months (treatment duration 6 months);

The study is being conducted in Chennai and Madurai. The estimated sample size for this trial is 1650 patients; so far 200 patients have been enrolled to the trial.

The study is registered in the clinical trials registry of India. (PROVCTRI/2008/091/000024)

[Contact person: Dr.M.S.Jawahar (E-Mail ID: jawaharms@trcchennai.in)]

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

(Funded by Department of Biotechnology, India)

Background

The immunomodulator containing *Mycobacterium w* was developed by the National Institute of Immunology, New Delhi in 1980. It has been found to be useful in the prevention of TB in experimental animals. A pilot study conducted to evaluate the role of *Mycobacterium w* in improving sputum conversion rate in pulmonary TB, showed that the conversion rate was faster when *Mycobacterium w* was added to the short course chemotherapy. Immunomodulators work against persistors, which may result in reducing the relapse rates. The addition of immunomodultor 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 the addition of the *Mycobacterium w* vaccine to standard anti-TB drugs

Methods

This study was being initiated by the Department of Science and Technology and was planned as 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. Though it was initially proposed to admit 128 patients to the study, this number was restricted to 60 patients by the funding agency.

Results

The study was initiated in March 2006. In the two year period till March 2008, 268 patients were registered, 115 screened and 59 enrolled into the study. Vaccine acceptability has been good among these patients. Of the 59 enrolled, 28 have completed treatment and are on follow up, 19 are still on treatment, 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. Four patients required change of chemotherapy – 2 due to multidrug resistance, 1 due to clinical complication, and one due to pregnancy. Two patients who had hepatic adverse events also had change of chemotherapy. This study is ongoing.

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

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

Background

TRC has been doing the drug resistance surveillance of patients treated with RNTCP regimens in one TB unit in Tiruvallur district in Tamil Nadu, south India (1999 to 2005). There were 52 patients who failed to Category-II regimen. The drug susceptibility testing (DST) results showed that 32% had multi-drug resistant TB (MDR-TB), 26% had drug resistant organisms but were not MDR-TB (resistant to INH/Sm/SmINH/EMB), 29% harboured fully susceptible organisms, and in 13% there was no growth in the culture.

DOTS plus using standardized Category-IV regimen is being implemented in India in a phased manner. There is no report, on the feasibility and effectiveness and adverse reaction of the Category-IV regimen.

Similarly there is no guideline on the management of patients who fail to Category-II but are non MDR.

Aim

- To assess the feasibility, effectiveness and adverse reactions of directly observed short-course (DOTS) plus regimen compared to modified DOTS plus regimen for patients who fail to Category-II regimen and have MDR-TB

Methods

Management of patients with MDR-TB

Eligibility criteria: A patient whose TB is due to bacilli resistant to at least INH and RMP with or without resistance to other drugs will be eligible for the study. At least one sputum smear examined within 10 days of starting treatment should be positive for AFB.

Treatment regimens: Patients have been randomly allocated to one of the following regimens based on the drug resistance pattern (stratification based on resistance to first-line drugs alone or resistance to one or more of any second-line drug along with MDR-TB)**.**

Regimen I: 6(9) (K, Of, Eth, Z, E & Cy) 18 (Of, Eth, E & Cy)

Regimen II: 6(9) K_3, (Of, Eth, Z, E & Cy) 18 (Of, Eth, E & Cy)

Regimen I: It is a standardized treatment regimen approved by RNTCP (Category-IV regimen - DOTS-Plus) for the treatment of MDR-TB. It consists of an intensive phase of 6 drugs for a period of 6-9 months followed by a continuation phase of 18 months of 4 drugs.

Regimen II: Similar to regimen I but Kanamycin given three days a week instead of daily.

Treatment duration

All patients from Tiruvallur district are being hospitalized for the first 2 - 4 weeks of treatment. Later, they get discharged, with a 7-day supply of drugs including kanamycin. Treatment is being arranged from the nearest Primary Health Centre (PHC) as directly observed treatment (DOT).The drugs are supplied from TRC on a monthly basis. All patients from Chennai Corporation are being given treatment as DOT from TRC or the subcenters by TRC staff.

Sample size:

It has been planned to recruit about 75 patients to each regimen.

Clinical and laboratory investigations:

Before starting treatment patients undergo detailed history elicitations for previous second line ATT, chest X-ray, haemogram, liver and kidney function tests, pregnancy test for female patients, 3 sputum examinations for smear and culture for first and second line anti-TB drugs and enzyme linked immunosorbent assay (ELISA) for HIV antibodies.

After starting treatment patients are being followed up every month and investigations repeated periodically. Patients are being monitored closely for adverse reactions.

Outcome measures: The following outcome measures will be analysed:

- Sputum smear conversion at 3 and 6 months of treatment
- Sputum culture conversion at 3 and 6 months
- Favourable bacteriological response at the end of treatment
- Adverse reactions to anti-TB drugs

Treatment failures will be managed by individualized regimens based on the DST result.

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

Management of patients not having MDR-TB

Background

Based on the DST results, patients who do not have MDR-TB are being allocated to one of the two regimens suggested below. Patients harbouring fully

susceptible bacilli or having resistance to INH/Sm/SmINH/EMB come under this group.

Aim

- To assess the treatment outcome of a re-treatment regimen for patients who fail to Category-II regimen and not having MDR-TB based on drug sensitivity test

Regimens

Patients are allocated to one of the two regimens (stratification based on sensitivity to INH).

Regimen I : **6 K(REZ) 3 (REZ)**

Regimen 2 : **6 K(REZH) 3 (REZH)**

Sample size

It has been planned to recruit about 75 patients to each regimen.

Assessments and follow up are similar to patients having MDR-TB.

Outcome measures: The following outcome measures will be analysed:

- Sputum smear conversion at 3 and 6 months of treatment
- Sputum culture conversion at 3 and 6 months
- Favourable bacteriological response at the end of treatment

Treatment failures are being managed based on the DST result.

Intake to the study was initiated in September 2007 and upto 31st March 2008, 24 patients have been recruited (17 in MDR and 5 in non MDR).

The study is in progress.

[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 pulmonary TB cases is vital as these cases 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 required treatment develop active disease with in the first three months.

Aims

Primary objectives

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

Secondary objectives

- To study the proportion of TB patients among this group (confirmed by culture) and their correlation with chest X-ray finding
- To obtain information on the etiological profile of respiratory infections and their sensitivity pattern and appropriateness of antibiotic algorithm

Methods

Patients referred with cough of ≥3 weeks and 3 smears negative to AFB are registered. The patients undergo 3 sputa examined for AFB by smear and culture and chest X-ray. They get randomly allocated to one of the following regimens for 10 days:

- Co-trimoxazole (sulphamethaxozole-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, X-ray and sputum examinations are repeated and patients are assessed for persistence of symptoms. If repeat sputum was negative by smear and if X-ray was suggestive of TB they are started on category-III regimen. If X-ray was abnormal but not suggestive of TB they are followed up for 6 months with monthly sputum examination.

All the patients are being reviewed with culture results. It is proposed to admit 700 patients to each antibiotic arm. Till 31st March 2008, 180 patients have been recruited.

The study is in progress.

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

Evaluation of a diagnostic algorithm for HIV-positive TB suspects who are initially smear negative

(In collaboration with NARI, Pune)

Background

In HIV-infected patients with active TB disease, sputum smears are more likely to be negative for AFB by smear microscopy. The RNTCP bases diagnosis of TB on sputum smear examinations and treatment consists of a course of antibiotics.

Aim

- To determine the utility of initial chest X-ray and sputum culture (LJ solid media) in the diagnostic algorithm for TB among HIV-infected initially smear negative TB suspects

Methods

This has been planned as a multicentric, prospective study which will enroll 540 HIV-infected patients with suspected TB disease. Those suspects who are smear negative on initial sputum examination have a chest X-ray and sputum culture performed, receive a course of broad spectrum antibiotics, and are reviewed with a repeat chest X-ray after 15 days. Patients considered seriously ill or with chest X-ray suggestive of TB have ATT started by the site physician.

Results

Interim analysis of 114 patients (87 males, 27 females) was done. Their mean age and body weight (SD) were 36±9 years and 47±9 kg respectively. The median CD4 cell counts were 191cells/mm^3. Eighty one percent of the patients had cough and breathlessness for >3 weeks, 60% had fever for >2 weeks and 89% had weight loss. 21 patients (19%) had a positive TB culture initially. 76 patients (62%) had an abnormal chest X-ray, of whom 25% had a positive initial culture. Of the 76 patients with an abnormal chest X-ray, 34 (45%) demonstrated clinical and radiographic improvement after treatment with antibiotics.

The study is ongoing and enrollment will be completed in 2008.

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

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

(Collaborative study with National Institute of Health, USA)

Background

The goal of this study is to determine the changes in HIV viral loads that occur in patients co-infected with HIV and filaria, over 1 year following treatment with DEC/Albendazole and to compare those with changes in viral loads among HIV-infected patients without filarial co-infections. The total sample size required for this study is 138 (HIV/Filarial–46, HIV alone–92). Two groups of patients are recruited to the study. The first group comprises of patients with HIV and filarial infection (detected by serum antigen test). The second, or control group consists of patients with HIV infection, but not filariasis. The second group of patients is being matched to the first group based on age, gender, HIV viral load and CD4 count. Patient recruitment is being done in both TRC and YRG. Screening of patients for this study started in May 2007 at TRC; up to March 2008, 156 patients were screened, of whom 23 (positives–7, controls–16) patients have been were recruited to the study.

The study is ongoing.

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

SOCIOLOGICAL RESEARCH

Completed Studies

Psychosocial and demographic predictors of HIV sexual risk and HIV infection in men who have sex with men in Chennai, India

Collaborative study with Harvard University, USA

Background

Men who have sex with men (MSM) in India are stigmatized, understudied, and potentially at high risk for HIV. The impact of psychosocial issues facing this hidden population on HIV risk behavior and HIV infection can help shape culturally relevant HIV prevention interventions.

Aim

- To study the psychosocial and demographic predictors of HIV sexual risk and HIV infection in MSM in Chennai, India

Methods

Outreach workers recruited 210 MSM in Chennai who completed an interviewer-administered psychosocial assessment battery and underwent HIV testing and counseling.

Results

Twenty two percent (46/210) of the sample reported any unprotected anal intercourse in the past three months, 8% (16/202) tested positive for HIV and 26% (55/210) had previously participated in an HIV prevention intervention. The mean age was 28.9 years (SD=7.83); MSM subpopulations included Kothi (25.7%), Panthi (37.6%) and Double-decker (36.7%). In a multivariable logistic-regression model controlling for age, MSM subpopulation, marital status, and religion, significant predictors of any unprotected anal intercourse included education (adjusted OR=.54; p=0.009; such that more education was protective), not having previously participated in an HIV prevention program (adjusted OR=3.75; p=0.05), depression (adjusted OR=2.8; p =0.02), weekly alcohol use (adjusted OR=3.56; p=0.07), and self efficacy (adjusted OR=.40; p<0.0001; such that higher self efficacy was protective). In a multivariable logistic regression model controlling for age, MSM

subpopulation, marital status, and religion, significant predictors of testing positive for HIV infection were: education (adjusted OR=0.53; p=0.05; such that more education was protective) and not currently living with parent(s) (adjusted OR=3.71; p =0.05).

Conclusions

Given the high prevalence of HIV among MSM and relatively low rate of participation in HIV intervention programs, efforts to reach hidden subpopulations of MSM in India are still needed. Such programs for MSM in India may need to address co-occurring psychosocial problems faced by this population to maximize their chances of reducing risk.

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

Gender difference in sexual behavior among people living with HIV in Chennai, India

Background

Risky sexual behaviour is usually the focus of HIV prevention programmes and little attention has been given to sexual behavior patterns among HIV-positive individuals. In order to ensure that people with HIV receive high quality sexual and mental health services, providers must have a comprehensive understanding of the issues and challenges faced by men and women with HIV.

Aim

- To gain insight into the gender differences in sexual behavior patterns among seropositive men and women

Methods

This was planned as a descriptive cross sectional study on a cohort of 203 seropositive patients (102 women and 101 men) attending outpatient clinics in the TRC and the STD clinic of the Government General Hospital, Chennai.

Results

Fifty three percent of the women were discontented with the sexual relationship with their spouse as compared to 23% of the men ($p<0.001$).

Thirty two of the 54 women who refused sex said that their spouses reacted violently to their refusal. More men than women reported to having extramarital relationships most often with a commercial sex worker or a friend, without condoms and usually under the influence of alcohol.

Conclusions

There are gender differences in sexual behaviour patterns among men and women. Understanding these differences is important to plan gender based intervention strategies in order to ensure that people living with HIV have a better quality of life addressing their sexual concerns both within and outside of marriage. The findings will also help in advocacy and prevention programmes aimed at HIV-AIDS control.

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

Ongoing Studies

Health seeking behavior and awareness of TB among migrants – brick kiln workers - a study from Tiruvallur district, Tamil Nadu, India

Background

The Model DOTS project was implemented in the Tiruvallur district, Tamil Nadu to study the feasibility and impact of the RNTCP. During the study it was found that there was a fairly large number of brick kiln workers who were a migrant population in this area. There was dearth of information on their health seeking behaviour and management of TB. A study was therefore done to find out the health seeking behavior and awareness of TB among this group.

Methods

It was planned to conduct the study in two phases. The first phase was to do a qualitative situation analysis of the profile of brick chambers and the brick kiln work structure. The list of brick kilns was obtained from the district collectorate. They were mapped geographically and a random sample was chosen. There are 116 villages with 450 brick kiln cambers. The investigators met the owners of the brick kilns at their general body meeting which is conducted once in 3 months. They were briefed about the study and the importance of the information obtained to help in the health care and management of their workers who could be diagnosed with TB, before the initiation of the study. The group offered their full cooperation.

A sample of 80 villages was covered with chambers. Attempts were made to contact the brick kiln workers, owners and other chamber staff which included supervisors or accountants. A semi structured interview guide to ensure uniformity of questions was prepared with questions pertaining to the health problems usually faced by the workers, the health care facilities accessed and nature and period of work

Findings of the situational analysis

Tiruvallur district is one of the 33 districts in Tamil Nadu and houses a large number of the brick chambers due to the nature of soil that is suitable for bricks (Census 2001). Each chamber has around 100-150 workers. They are usually from Madurai, Villupuram and Thiruvannamalai. There were a few chambers which comprised exclusively of

workers from Andhra Pradesh and Orissa. The period of stay was usually between January to June and the workers stayed in one chamber for approximately 6 months after which they went back to their original place of stay. The majority of brick kiln workers accessed private care facilities for health related problems. One of the major reasons for this was that the brick kiln owners have an understanding with these private practioners and their consultation fees and medicines are paid by the owners. There was no policy for treatment of those with symptoms of TB or diagnosed with TB. The brick kiln owners said they had no problems in employing workers with TB or diagnosed with TB after being recruited by them and were also willing to have a DOTS centre in their brick kiln cambers. It is important therefore to gain more information of the profile of chest sympomatics**,** TB patients and their TB management if diagnosed with TB as this is a population that is mobile. The second phase of this study to cover these aspects is being planned.

[Contact person: Mrs.K.J. Jagannatha Rao (E-Mail ID: jaggarajamma@trcchennai.in)]

Community-based approach to designing an AIDS program for HIV-positive mothers in India

Collaborative study with the UCLA - University of Los Angeles, USA

Background

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 increase. Mothers living with HIV-AIDS (MLH) carry a triple burden of being HIV-infected, are mothers of children who may or may not be positive themselves, and care givers to their HIV-infected spouses. These burdens pose huge challenges for MLH and need to be addressed.

Aim

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

Methods

This was planned as a qualitative study utilizing focus groups consisting of HIV-infected mothers recruited from a large maternity hospital and STD clinic in Chennai, India. Each group comprised of 5-7 participants. The discussions are carried out using a semi structured focus group guide, Content analysis is done to determine common themes discussed among the groups. So far, 7 focus group discussions have been done.

Results

Some of the interim findings expressed are discrimination by physicians and other health care workers which have been a major impediment expressed by MLH in accessing quality health care. Concerns around disclosure have also been expressed. The study is ongoing

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

A study of the care seeking behaviour of persons with chest symptoms from rural and urban areas in Tamil Nadu after implementation of the RNTCP

(Funded by Model Dots Project-8th year plan)

Background

The TB control programme is based on passive case finding. It is therefore crucial to understand the health care seeking behavior of chest symptomatics to understand how they respond to their symptoms, their first point of consultation, delay in seeking care and problems faced if any. Hence a study was carried out prior to the RNTCP by the TRC. One of the main findings of this study was that the first point of consultation was a private health care facility and the patient shifted to other facilities when dissatisfied. After the implementation of the RNTCP and the accessibility and availability of drugs at public health facilities, we expected that there would be a change in the health care seeking behavior patterns of chest symptomatics.

Aim

- To find out the health care seeking pattern of the persons with chest symptoms after implementation of the RNTCP

Methods

This study is conducted in two urban and two rural communities in Tamil Nadu, south India. Households are selected from the randomly selected streets. The heads of the selected households are contacted to find out “persons with chest symptoms”. It is proposed to cover a total of 600 chest symptomatics, 150 in each of the four communities.

The study is ongoing.

[Contact person: Mrs. Niruparani Charles (E-Mail ID: nirupa@trcchennai.in)]

Perceptions of HIV-positive individuals on disclosure of their HIV status to their children

Background

With the introduction of ART and the need for life long treatment, HIV-infected parents are faced with the biggest challenge on how to disclose their HIV status to their children. Although HIV disclosure serves as a stressor, there is some evidence that suggests that it facilitates emotional support, which may lead to more effective coping and enhanced psychological adaptation. However, the fear of stigma and discrimination by the children of people living with HIV/-AIDS (PLHAs) may inhibit people from disclosing their HIV status.

Aim

- To study the perception of HIV-positive individuals on disclosure of their HIV status to their children

Methods

This cross sectional study is being carried out covering PLHAs attending the out patient TRC clinics in General Hospital and Chetpet after obtaining their consent. In-depth interviews are done using a semi structured interview schedule. So far, 75 patients have been recruited.

The study is ongoing.

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

A study on sexual behaviour among sero-discordant individuals

Background

There is dearth of information in India on sexual behavior and sexual risk factors among HIV sero-discordant couples. This could help understand the sexual behavior patterns and sexual risk factors for HIV transmission which could be useful for health care providers in dealing with this group of individuals.

Aim

- To explore the sexual behaviour patterns among sero-discordant individuals

Methods

In-depth interviews were conducted among eligible patients after obtaining their consent. Patients were recruited from outpatient clinics of TRC and TRC subcentres at Government Hospital and Vellore unit. So far, 70 interviews have been conducted.

The study is ongoing.

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

Parental and care givers perceptions on disclosure of HIV diagnosis to seropositive children

Background

One of the most difficult challenges for health care providers dealing with HIV-AIDS is dealing with seropositive children. The challenges vary from diagnosis, administering drugs, nutrition and most of all, how to disclose the HIV diagnosis to their children. This includes how and when to disclose, whether to disclose, who needs to disclose and how to deal with the problems after disclosure. In order to answer these questions, this study is being done as part of a clinical observational pediatric study done at the TRC.

Aim

- To study the perception of care givers on disclosure of HIV diagnosis to seropositive children

Methods

Children are referred to the TRC clinics from various government hospitals, institutions and NGOs dealing with children. The children are accompanied by either their parents or care givers depending on where they come from. The respondents for this study are

either the parents or the care givers who are enrolled to the study after obtaining their consent. A semi-structured interview schedule is being used to elicit data. So far, 50 care givers have been interviewed.

The study is ongoing.

[Contact person: Mrs. Meenalochani Dilip (E-Mail ID: meenudilip@trcchennai.in)]

A study on quality of life among HIV-TB patients on ART

This study is part of a controlled clinical trial to evaluate the safety and efficacy of two different once daily ART regiments along with ATT in patients with HIV-1 and TB. All patients enrolled to this study are considered eligible if they consent to being a part of the study. The WHO-QOL BREF questionnaire is used to measure quality of life at 0, 1 year and 2 year intervals. The study will throw light on factors that influence QOL among HIV-TB patients on different regimens of ART. So far, 47 patients have been enrolled.

The study is ongoing.

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

VACCINE TRIAL

Phase I trial of a HIV vaccine in healthy volunteers

This study initiated in 2005 was designed to test the safety and tolerability of a recombinant HIV vaccine in a Phase I double-blind clinical trial. A total of 32 volunteers were recruited and were given 3 injections of the vaccine or the placebo at two dose levels (12-low dose; 12-high dose; 8-placebo). The volunteer visits have been completed and no adverse or serious adverse events pertaining to the study product were noted. The immunogenicity data is being analysed now.

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

EPIDEMIOLOGICAL & OPERATIONAL RESEARCH

Epidemiological impact study: Disease survey

Background

Directly observed treatment short-course (DOTS) was implemented in Tiruvallur district of Tamil Nadu in May 1999. To assess the epidemiological impact of DOTS strategy, TRC is carrying out a series of sample surveys with 2½ years duration between surveys to estimate the prevalence of disease in this district, covering a population of 5,80,000.

Aim

- To study the trends over time for disease and thereby to measure the impact of DOTS strategy in this region

Methods

All adults aged ≥15 years included for the disease survey was screened by two screening methods namely, elicitation of symptoms and X-ray examination. Two sample of sputum specimens were collected from those who were either symptomatics and/or abnormal X-ray suggestive of TB. These specimens were processed for smear and culture and those who became bacteriologically positive were referred for ATT if they satisfied the RNTCP guidelines.

Results

Three serial disease prevalence surveys were already completed. The fourth survey was started in June 2006 and is in progress. Coverage in the survey was above 90% for all examinations, namely, symptoms, X-ray and sputum examination. The coverage upto March 2008 is shown in table 7.

Table 7: Coverage for examinations – Fourth survey (till March 2008)

Activities	Fourth survey
Enumeration	69133
Symptom screening	64076 (93%)
X-ray screening	62918 (91%)
Sputum eligible	8085
Sputum collection	7818 (95%)

A total of 266 Individuals were identified as cases through examination either by smear, culture or both.

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

External quality assessments for sputum smear microscopy in the laboratory network

Background

RNTCP is a DOTS based programme and sputum smear microscopy is the diagnostic technique used. The quality of services in the microscopy centres needs to be constantly evaluated and assured. The External Quality Assurance (EQA) protocol is implemented throughout the country which includes onsite evaluation, panel testing and random blinded cross-checking that are done to evaluate the performance.

Aims

- To review the Intermediate Reference Laboratory (IRL)-EQA activities in 19 districts in India
- To conduct National Reference Laboratory-Onsite evaluation (NRL-OSE) visits focused on identifying the operational and technical problems in smear microscopy

Methods

NRL-Onsite evaluation visits

TRC conducted NRL-OSE visits in 9 states during the year 2007. It reviewed the problems that were identified and corrective actions were suggested. Some of the corrective suggestions made for IRL and districts during the year are given below:

IRLs

- All the IRLs have started functioning well. But all the equipment supplied for culture and drug sensitivity in some IRLs needs replacement or repairs

- In IRL Goa, appointment of a microbiologist and 2 Laboratory Technicians should be done and trained in RNTCP, EQA as well as in culture and Drug susceptibility testing procedures.
- Record maintenance for internal quality control at State TB Training and Demonstration Centre, Gujarat should be done properly.
- Guidelines on fluorescence microscopy recently published by Central TB Division should be followed for grading smears and for internal quality control

District

Most of the operational and technical problems were identified at the district level.The following suggestions was made for districts:

- Proper glasswares should be made available at the District TB Centres, for the bulk preparation of reagents wherever required
- Random blinded rechecking (RBRC), coding and reporting should be carried out under the supervision of the District TB Officer
- All the newly appointed technicians should be trained in RNTCP-EQA activities
- Disposal pits were posing a problem during rainy seasons. This needs to be addressed.
- District TB Officer should be motivated to take interest in the programme and supervise the activities

More emphasis is given on the RBRC data and correcting the system whenever there are errors in the RBRC. At the end of OSE visits, the summary reports were made based on observations. This report was submitted to the IRL Director, State TB Officer for taking corrective actions. The action taken reports were obtained from the states within one month after the visit. Of the 131 suggestions provided to nine IRLs for corrective action, 104 (79%) were implemented within a month.

Panel testing

For the year 2007, 5 (9.4%) out of 53 persons from 7 states made 6 errors (includes Microbiologist-1, Laboratory Technician-1 and Senior TB Laboratory Supervisor -3) in panel testing

RBRC activities at state level

RBRC data available from 8 states under TRC indicated that some of the districts were completely devoid of errors while some districts reported high and low false errors (table 8).

Table 8: State-wise RBRC activities

S.no	State	Districts	DMC	Sample size	HFP	HFN	LFP	LFN	QE	Total Error
1	Andhra Pradesh	24	893	123234	62	133	34	65	127	**421**
2	Goa	2	18	3047	2	5	0	2	4	**13**
3	Gujarat	29	694	75812	29	125	29	138	77	**398**
4	Kerala	14	457	101307	32	87	86	89	38	**332**
5	Punjab	20	285	3484	5	43	21	26	103	**198**
6	Sikkim	4	20	2972	1	3	1	1	4	**10**
7	Tamil Nadu	30	782	27751	24	57	8	33	41	**163**
8	UP-Lucknow	31	717	78171	135	198	131	138	367	**969**
9	UP-Agra	35	711	84939	169	265	101	119	262	**916**
Total		**189**	**4577**	**500717**	**459**	**916**	**411**	**611**	**1023**	**3420**

Conclusion

On site evaluation is important to ensure quality of sputum microscopy. NRL suggestions resulted in immediate corrective actions and had a sustained impact. OSE and RBRC supervision at the district level needed regular monitoring for improvement and sustenance of the program.

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

The impact of HIV infection on recurrence of TB

Background

Molecular typing of the strains of tubercle bacilli is a reliable method available today to determine whether the patient's relapse is due to exogenous or endogenous reactivation. For decades, the issue of the role of exogenous re-infection versus endogenous reactivation has been debated. We have earlier shown from studies conducted in TB patients not infected with HIV, endogenous reactivation predominates. However there is no data with regard to HIV-TB patients from India. Specific phenomenon such as emergence of HIV related TB co-infection could modify the evolution of TB transmission. Hence in this study we have compared the molecular epidemiology of HIV-TB co-infection with non HIV-TB using multiple tools.

Aim

- To study the rate of exogenous re-infection and endogenous reactivation in HIV-TB patients compared with non HIV-TB patients by directly comparing the fingerprints of pre and post treatment isolates using three genotyping markers

Methods

Twenty five HIV-TB patients and 23 non HIV-TB patients were included in this study. Three sputum samples at the start of the treatment, during treatment and 2 sputum samples from the follow up period were collected. Smear and culture was done on the Lowenstein-Jensen medium (LJ). DNA from *M tuberculosis* cultures was extracted by standard CTAB-NaCl extraction method.

Molecular typing was done by the following three methods:

- IS6110 RFLP
- Spoligotyping
- MIRU-VNTR genotyping

Clustering by the different typing methods was analyzed by using Bionumerics (Applied Maths).

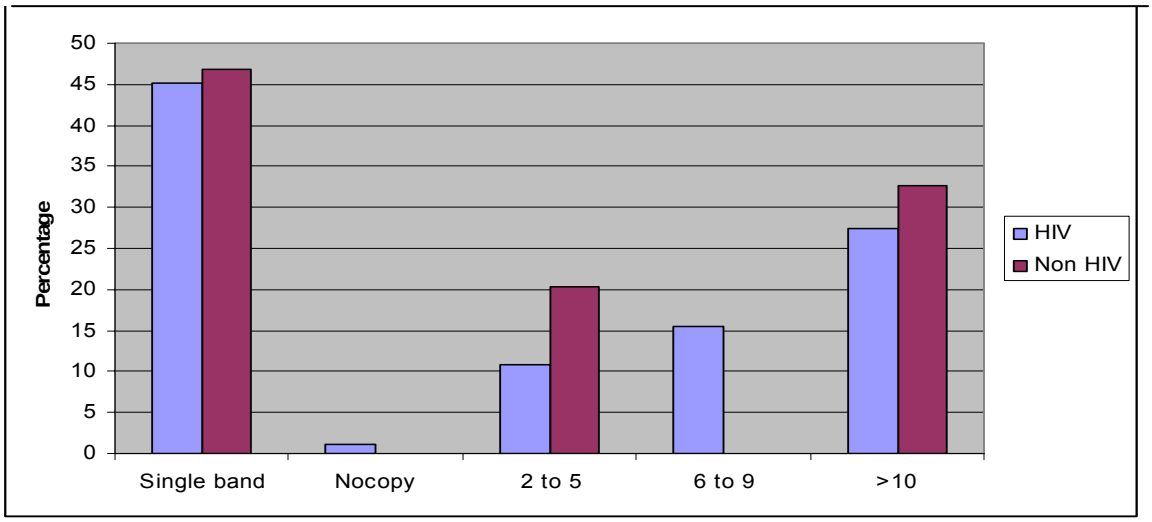
Results

The major finding of the study was that HIV patients recurred by a higher percentage of exogenous re-infection compared to non HIV-TB patients who relapsed by endogenous reactivation. IS6110 RFLP data revealed that both HIV patients and non HIV patients were infected by higher percentage (45.1 and 46.9% respectively) of single copy *M. tuberculosis* isolates. Spoligotyping data of HIV-TB and non HIV-TB patients in whom TB reoccurred showed that *M. tuberculosis* isolates of EAI3 Clade predominantly infected HIV-TB patients (HIV-TB 48.9% and non HIV-TB 38%) whereas *M. tuberculosis* isolates of EAI5 Clade predominantly infected non HIV-TB patients (HIV-TB 17.8% and non HIV-TB 26%) (Fig.1) Beijing strains infected HIV-TB patients to a higher degree than non HIV-TB patients.

Conclusion

The high recurrence rate of TB in HIV-positive patients could be reduced by intensification of therapy. Since TB infection in an HIV-positive individual does not seem to confer immunity to individual, continuous prophylaxis is a better choice. Conversely since the risk of infection is low in non HIV-TB patients the prospect of vaccine in these patients is promising.

Fig.1: RFLP of HIV and non-HIV isolates



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APPLIED RESEARCH

Completed studies

Studies on sputum transported in cetyl pyridinium chloride for the detection of *M. tuberculosis*

Background

In drug resistance survey, sputum samples from pulmonary TB patients are collected in cetyl pyridinium chloride **(**CPC) solution and transported to the reference laboratory for bacteriological investigation. Smears from sputum transported in powder CPC (P-CPC) yielded less acid fast bacilli positivity. It is important to understand the reasons for the poor yield, since it is easier to transport sputum in P-CPC.

Aim

- To elucidate the reasons for the reduction of AFB positivity in sputum transported with P-CPC

Specific objectives

- To detect and demonstrate any cell wall changes after exposure to CPC using transmission electron microscopy (TEM) and phage adsorption assay
- To estimate the proportion of metabolically active cells of *M. tuberculosis* after exposure to CPC using fluorescein diacetate-ethidium bromide (FDA-EB) vital staining method and viable count
- To determine changes in mycolic acid content of tubercle bacilli exposed to CPC using high performance liquid chromatography (HPLC).

Results and conclusion

Damage to cell wall after exposure to CPC was demonstrated by both TEM and phage adsorption assay. FDA/EB stain showed upto 90% viable cells (green) prior to CPC exposure and upto 70% non-viable cells (red-orange) after CPC exposure. Viable count was not altered significantly after exposure to CPC.

HPLC analysis revealed marked reduction in mycolic acid content. To conclude, P- CPC can be used to transport the sputum for culture of *M. tuberculosis.*

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Effect of rifampicin, isoniazid, pyrazinamide and ethambutol on the steady state pharmacokinetics of moxifloxacin

Background

Moxifloxacin (MFX) is reported to have promising antimycobacterial activity, and has a potential to shorten TB treatment. Moxifloxacin undergoes phase II metabolism by means of sulphate and glucuronide conjugation. Rifampicin (RMP), a potent inducer of cytochrome P-450 isoenzymes also induces the phase II glucuronidation pathway.

Aim

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

Methods

The study was performed in 24 healthy adults who were not suffering from any illness. Each subject was investigated on two occasions; a cross-over design was employed in which each subject served as his control. A baseline pharmacokinetic study of MFX (400 mg once daily) was conducted (Occasion 1) and repeated after one week of daily MFX with either RMP (450/600 mg) or INH (300 mg) or PZA (1500 mg) or EMB (1200 mg) (Occasion 2). Each drug group had six subjects. During both occasions, serial blood samples were collected pre-dosing and at 1, 2, 4, 6, 8 and 12 hrs after drug administration. Plasma MFX concentrations were determined by a validated HPLC method.

Results

The pharmacokinetic variables of MFX alone and in combination with RMP, INH, PZA and EMB are shown in table 9. Plasma MFX concentrations were significantly lower when co-administered with RMP; the predosing levels were below the minimal inhibitory concentration (MIC) (0.50 µg/ml) in 2 out of 6

subjects in the presence of RMP. Plasma exposure of MFX was significantly lower when combined with RMP ($p<0.05$). This was accompanied by a significant increase in the plasma clearance of MFX ($p<0.01$). Although peak concentration of MFX was lower when combined with RMP than when given alone (4.25 vs. 6.36 μg/ml), the difference was not statistically significant ($p=0.074$). Administration of INH, PZA and EMB did not appear to have any significant effect on the plasma concentrations of MFX. The arithmetic mean ratios of exposure of MFX when given alone to that combined with RMP, INH, PZA and EMB were 0.61, 0.76, 1.00 and 1.01 respectively.

Conclusions

Concomitant RMP administration resulted in a 39% mean decrease in the plasma exposure and a 46% mean increase in the oral clearance of MFX. It is uncertain whether the decrease in MFX concentration observed in this study would affect its treatment efficacy. Further studies on patients with TB are needed to determine the clinical relevance of the MFX-RMP interaction.

Table 9: Steady state pharmacokinetics of MFX alone and in combination with RMP, INH, PZA & EMB (Mean ± SD)

Drugs	Mean ± SD				
	C_{max}	T_{max}	$AUC_{(0-12)}$	Cl	$t_{1/2}$
MFX (n = 6)	6.36 ± 1.96	3.0 ± 1.10	51.75 ± 13.45	4.86 ± 1.08	8.49 ± 2.45
MFX + RMP	4.25 ± 0.48	2.0 ± 1.10	31.43* ± 4.88	8.96* ± 1.64	6.71 ± 1.45
MFX (n = 6)	6.19 ± 1.85	2.33 ± 1.37	50.09 ± 16.8	5.27 ± 1.67	8.25 ± 0.73
MFX + INH	5.06 ± 0.72	1.83 ± 1.17	37.97 ± 6.03	6.32 ± 1.34	8.61 ± 1.04
MFX (n = 6)	4.38 ± 0.79	1.83 ± 0.41	35.46 ± 6.00	6.96 ± 1.39	8.69 ± 1.16
MFX + PZA	4.54 ± 0.91	2.00 ± 1.10	35.51 ± 7.88	7.59 ± 3.00	8.16 ± 2.27
MFX (n = 6)	4.62 ± 0.84	2.00 ± 1.10	35.02 ± 2.82	7.27 ± 1.53	7.98 ± 3.15
MFX + EMB	4.40 ± 0.26	2.50 ± 1.22	35.53 ± 2.40	6.37 ± 0.47	9.05 ± 1.02

$P < 0.05$ vs. MFX alone
C_{max} – Peak concentration; T_{max} – Time to attain C_{max}; $AUC_{(0-12)}$ – Exposure; Cl – Clearance; $t_{1/2}$ – Half-life

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

Single dose pharmacokinetics of lamivudine in healthy volunteers: comparison of blood and urine kinetics

Background

Lamivudine (3TC) forms an important component of highly active antiretroviral therapy (HAART) that is used to treat HIV-infected individuals in India. It is present in all fixed dose combination (FDC) pills, has a short elimination half-life,

and hence estimation of the drug in blood or urine could be useful in monitoring patient adherence to antiretroviral treatment.

Aim

- To study the single dose pharmacokinetics of 3TC in healthy subjects, and also to assess the correlation between plasma and urine kinetics of 3TC

Methods

Twelve healthy adult males meeting the study criteria were recruited to the study. The study was carried out at the Pharmacology Ward in Madras Medical College, Chennai. They were administered 3TC (150mg) under supervision and blood samples were collected pre-dosing and at 1, 2, 4, 6, 8, 12 and 24 hrs after drug administration. They were instructed to make complete urine collections excreted up to 24 hrs after drug administration. Lamivudine concentrations in plasma and urine were estimated by HPLC according to validated methods.

Results

The peak concentration of 3TC in plasma was achieved at about one hour, suggesting rapid and almost complete absorption. The concentration of 3TC at 24 hours was undetectable in all the 12 study subjects. The correlation between plasma exposure (0 to infinity) and percent dose of 3TC excreted in urine between 0 to 24 hrs was highly significant ($p < 0.001$; $r = 0.96$).

Conclusions

Urine 3TC was highly correlated with plasma exposure, which suggests that urine 3TC estimations can be used to obtain information on the bioavailability of the drug. Thus invasive blood collections can be replaced by simple, non-invasive urine collections. The study has also demonstrated the usefulness of plasma 3TC in predicting antiretroviral treatment adherence.

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Comparison of HPLC and spectrophotometric methods for estimation of antiretroviral drug content in pharmaceutical products

Background

Although the growth in antiretroviral availability is encouraging, it must be accompanied by independent quality-control studies to check for adherence of amount of active ingredient in the tablet/capsule to the stated content. The most common method used for analysis of antiretroviral drugs is by HPLC. However, many laboratories in resource-constrained settings may not afford to have HPLC equipment, which also requires skilled personnel to operate it. An alternative is to carry out drug estimations by spectrophotometry which is relatively cheap and simple.

Aim

- To compare the content of NVP, 3TC and stavudine (d4T) in single tablets/capsules estimated by HPLC and spectrophotometry methods

Methods

Twenty tablets/capsules each of NVP, 3TC and d4T were analysed for their drug content by HPLC and spectrophotometric methods. The tablets/capsule contents were crushed into a fine powder and suitably diluted in methanol to yield a concentration of 1.0mg/ml for each drug. Further dilutions were made using milli-Q water to obtain a final concentration of 25µg/ml. The stock solution (1mg/ml) of each drug was prepared by dissolving the pure powder in methanol and these solutions were stored at -20^{o}C.

Calibration curves containing known concentrations of each drug were prepared fresh individually and set up on each day by making suitable dilutions in water from the stock solution. The concentration curve was constructed for each drug using a set of calibration standards (12.5, 25 and 50µg/ml for NVP and 3TC and 6.25, 12.5 and 25µg/ml for d4T) that were prepared in water and run along with the unknown solutions with each run by HPLC and spectrometric methods.

HPLC method

Analysis was performed using C_{18} column. The mobile phase consisted of phosphate buffer and acetonitrile in different compositions for the three drugs. The UV detector was set at 260 nm. The retention times of 3TC, d4T and NVP were 3, 3.9 and 6.7 minutes respectively. The active ingredient in each

tablet/capsule was calculated from the height of the peak that was obtained on the chromatogram and compared with that obtained for the corresponding standard solutions of known drug concentration. Appropriate dilution factors were employed to calculate the drug content.

Spectrophotometric method

The instrument was set at 300, 285 and 270nm for NVP, 3TC and d4T respectively. The drug content in the unknown solutions was calculated based on the absorbance values of known standard solutions.

Prior to undertaking the drug assays, calibration standards of NVP, 3TC and d4T were run on six consecutive days by HPLC and spectrophotometric methods. The inter-day variability for each drug concentration was calculated and the linearity of the calibration standards was verified using estimates of correlation coefficient (r).

Results

The calibration curve of NVP, 3TC and d4T from six individual experiments for standard concentrations ranging from 12.5 to 50µg/ml for NVP and 3TC and 6.25 to 25µg/ml for d4T showed a linear relationship between peak height and drug concentration in the case of HPLC and absorbance and concentration in the case of spectrophotometric method. The NVP, 3TC and d4T content from 20 tablets are given in table 10. The percent variation between these methods ranged from 0.45 to 4.49% for NVP, 0 to 4.98% for 3TC and 0.35 to 8.73% for d4T.

Conclusions

The study suggests that the spectrophotometric method is as accurate as the HPLC method for estimation of NVP, 3TC and d4T in tablet/capsule. Hence laboratories that do not have HPLC equipment can also undertake these drug estimations using a spectrophotometer.

Table 10: NVP, 3TC and d4T content in tablets (mg)

	NVP (200mg)		3 TC (150mg)		d4T (30mg)	
	HPLC	Spec.	HPLC	Spec.	HPLC	Spec.
Mean (n=20)	208.9 (197.1 – 229.6)	208.1 (190.7 – 223.5)	151.2 (130.6 – 170.2)	151.9 (136.1 – 169.7)	28.6 (25.3 – 30.7)	28.6 (25.6 – 30.6)
% Variation	2.5 (0.45 – 4.49)		1.4 (0 – 4.98)		3.1 (0.35-8.73)	

Range is given in parentheses

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Improved diagnostic LRP assay for detecting active and non-replicating tubercle bacilli

Background

There is no simple, direct bacteriological tool available till date for the diagnosis of latent TB infection. Among the various alternative diagnostic tests being evaluated, tests based on mycobacteriophages have shown promise. The use of temperate mycobacteriophage Che12 and a temperature sensitive phage mutant, phAE159 (TM4 based) resulted in the development of the reporter phage constructs expressing FFlux driven by promoters of genes highly expressed during dormancy such as isocitrate lyase and alpha crystallin protein. TM4 based constructs expressing Fflux driven by alpha crystallin protein promoter exhibited detectable luciferase activity in dormant as well as in actively growing *M. tuberculosis* cells. Hence the present approach was aimed to evaluate the performance of the constructs in sputum samples.

Aim

- To evaluate the luciferase reporters in sputum samples for the development of a rapid diagnostic method for TB

Methods

Sensitivity of the constructs

M. tuberculosis H37Rv along with two clinical isolates (one of them is an MDR strain) were titrated with the LRP constructs to determine the minimal number of bacilli required to produce detectable light.

Evaluation of these constructs in sputum deposits

Evaluation of the lytic construct phAE129 in 50 sputum deposits, temperate construct phAETRC16 in 36 samples and the construct from *ts* mutant phAETRC201 in 18 sputum samples were carried out. The relative light units were measured at 4, 24 and 72 hours after the second infection.

Results and conclusion

When *M. tuberculosis* strains were titrated with the LRP constructs, phAETRC201 was more sensitive than other constructs. It was able to detect *M. tuberculosis* H_{37}RV as low as 81 cells while 4 logs more cells of clinical isolate were required to be detected by the same. Constructs phAETRC16 and phAETRC201, when tested in patients' samples, diagnosed almost all the positives detected by the conventional method. In addition, 17 more positives had been detected by LRP assay.

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Impact of antiretroviral treatment on nutritional and immunological status of HIV-infected children

Background

HIV infection is associated with growth retardation, immune deficiency and alteration in cytokine milieu.

Aim

- To investigate the impact of antiretroviral therapy (ART) on growth (BMI and plasma leptin) and immune response (CD4+ T cell counts, cytokine production, apoptosis and nitric oxide production) in HIV-infected children

Methods

The study population comprised of 11 HIV seropositive children starting on ART (mean age 5 years) and 12 HIV-positive children who did not require ART (mean

age 7 years). Both groups were followed up for 6 months. Height and weight were measured at each visit. CD4+ and CD8+ T-cells counts were analyzed by flow cytometry. Plasma levels of IFN-γ, IL-2, IL-10, IL-8, IL-6, IL-4, IL-5, IL-1, TNF-α and TNF-α were evaluated using a bead based flow cytometric assay. Plasma leptin, nitrate, nitrite and cell death were evaluated using commercially available ELISA kits. Statistical package SPSS (version 13.0) was used to analyze the data.

Results

The mean age of the children on ART was 5 years and that of the ART naïve children was 7 years. Following 6 months of ART, there was a significant elevation in body weight (13 vs. 15 kg), CD4+ T-cells percentage (13 vs. 24%) and plasma leptin levels (0.8 vs. 3.1ng/l). This was associated with a decline in plasma nitrate (0.8 vs. 0.4 mM/l), plasma nitrite (3.3 vs. 1.3 mM/l), IFN-γ (865$\pm$ 386 vs. 240$\pm$90 pg/ml), IL-6 (31$\pm$17 vs. 5$\pm$3 pg/ml) and apoptotic index (13$\pm$5 vs. 7$\pm$3) after 6 months of ART (table 11). On the other hand, there was no significant difference in any of the above parameters in children who did not receive ART, except an increase in weight.

Conclusion

The findings of the study imply that HIV associated immune activation and concomitant inflammation can be reduced by ART and this may have long term beneficial effects.

Table 11: Nutritional and immunological markers in HIV-positive children on ART and not on ART at baseline and at follow-up

Parameter	HIV-positive children on ART		HIV-positive children not on ART	
	Baseline	Follow-up (at 6 months of ART)	Baseline	Follow-up (at 6 months of ART)
Height (cms)	93 ± 5	101 ± 5	108 ± 5	111± 5
Weight (kg)	13 ± 1	16 ± 1	17 ± 2	19 ± 2
BMI	14 ± 1	16 ± 1	14 ± 0	15 ± 0
Plasma leptin (ng/ml)	0.9 ± 0.3	3.2 ± 0.9 *	1.8 + 0.7	2.3 + 0.4
CD4 T cell counts (cells/ml^3)	650 ± 152	1347 ± 332 *	1395 ± 224	1200 ± 200
IFN-γ (pg/ml)	865 ± 386	240 ± 90 *	312 ± 75	333 ± 93
Apoptotic index	13± 5	7 ± 3	7 ± 5	3 ± 2
IL-6 (pg/ml)	31 ± 17	5 ± 3 *	92 ± 6	94 ± 8
Nitrate (mM/L)	0.8± 0.2	0.4 ± 0.1 *	0.4 ±0.1	0.4 ± 0
Nitrite (mM/L)	3.3 ± 0.6	1.3 ±0.1*	1.33 ± 0.1	1.2 ± 0.1

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

Loss of CD27 expression on CD8+ T lymphocytes in HIV-infected pediatric patients

Background

Cytotoxic CD8+ T lymphocyte responses play a significant role in controlling viral infections. CD27 is a marker that has been utilized to distinguish memory (CD27+) from effector (CD27-) subsets of CD8+ T-cells.

Aim

- To characterize effector CD8+ T-cells in HIV-infected children

Methods

Seventy eight HIV-infected children (age ranging from 1.5-13 years) were recruited from the HIV/TB clinic of the TRC. CD27 expression on the CD4+ and CD8+ T-cell subsets was analyzed by dual color flow cytometry. HIV-1 plasma viral load was measured using the fully automated COBAS Amplicor HIV-1 monitor in 39 children. Intracellular IFN-γ production by HIV-1 p24 stimulated PBMC was also determined by flow cytometry.

Results

The mean percentage of CD4+ and CD8+ T-cells was 14.8 and 49.1% respectively. The mean viral load was 430,940 copies per ml of plasma (range 2020–750,000). The percentage of CD27+CD8+ cells was significantly higher than CD27-CD8+ cells (32$\pm$1.7 vs. 16$\pm$1.2, p<0.001) (Fig.2). Plasma viral load showed a positive correlation with the percentage of CD27-CD8+ T-cells (r=0.362, p<0.05) (Fig.3) and a negative correlation with percentage of CD27+CD4+ T-cells. CD27-CD8+ T-cells produced higher amounts of IFN-γ than CD27+CD8+ T-cells, in spite of the former being present at lower numbers than the latter.

Conclusion

The lower proportion of CD27-CD8+ cells in this cohort is suggestive of impaired maturation of CD8+ T-cells in chronically infected HIV-positive children, resulting in inefficient control of viral replication. Positive correlation between the proportion of CD27-CD8+ T-cells and plasma viral load indicates that the maturation of CD8+ T-cells is partially dependent upon antigenic stimulation. The effector CD8+ T-cell population was found to be responsible for most of the IFN-γ production.

Fig.2: Percentage CD4 and CD8 T-cell subsets in HIV-infected children

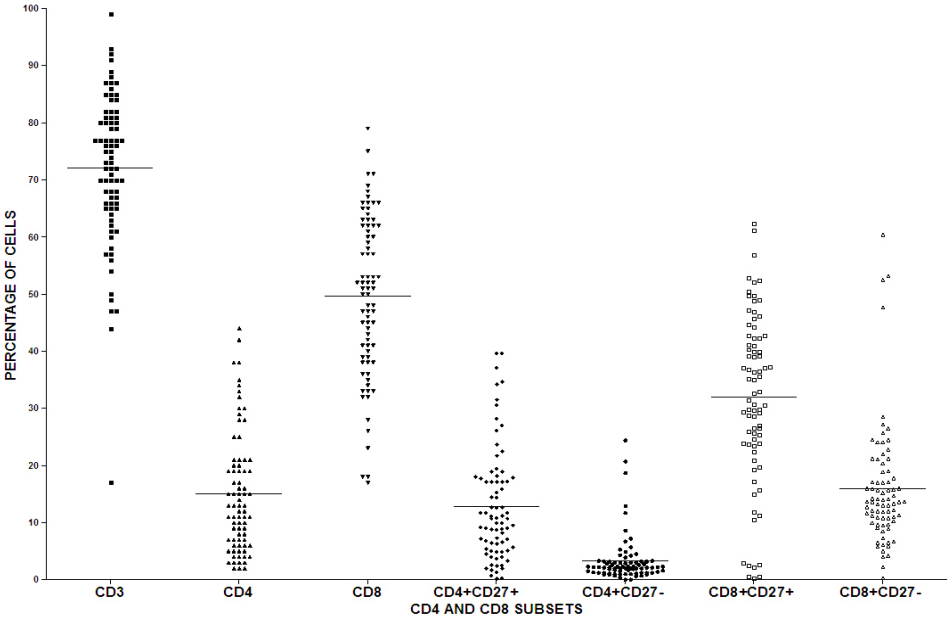
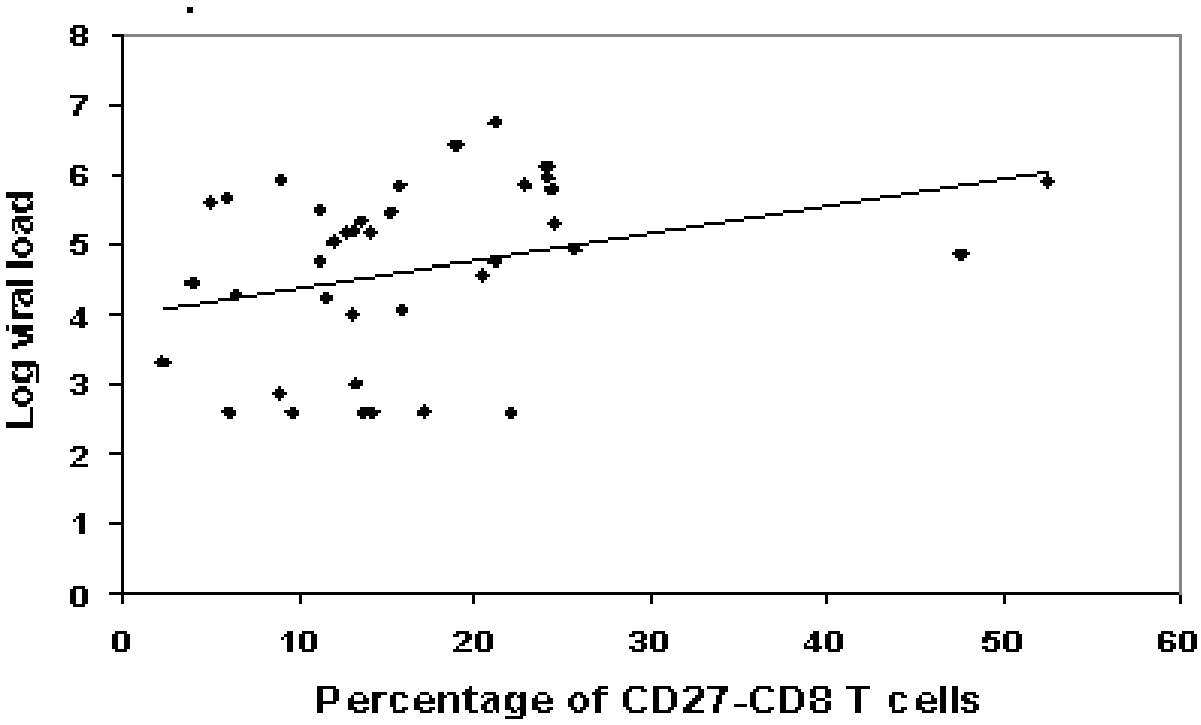


Fig.3: Correlation between CD27-CD8 T-cells and plasma viral load in HIV-infected children



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CD4/CD8 ratio as a surrogate marker for HIV infection in infancy

Background

It is estimated that 25-30% of HIV-infected infants will progress rapidly to AIDS/death in the first year of life. In the first 18 months of life serologic tests for HIV infection do not differentiate between exposure and infection due to maternally acquired antibodies. Virologic tests like DNA or RNA PCR are confirmatory but difficult to perform in resource-constrained settings.

Aim

- To evaluate whether CD4 count, CD4% or CD4/CD8 ratio could serve as a surrogate marker for HIV infection in infants below 18 months of age

Methods

The study included 273 infants with a mean age of 5.96 (range 0.2-18) months (139 females, 134 males). They were all born to HIV-positive mothers and were referred to the TRC between January 2006 and August 2008 for diagnosis. Informed consent was obtained from the parent. DNA PCR was performed using the Roche Amplicor HIV-1 DNA test (version 1.5) qualitative kit and CD4 and CD8 counts determined by two-colour flow cytometry (BD FACS calibur).

Results

Fifty five infants were DNA PCR positive while 218 were negative (table 12). There were significant differences in mean CD4%, CD4 count, and CD4/CD8 ratio between HIV-infected and uninfected infants. The mean CD4% and counts of PCR positive infants were significantly lower than those of the PCR positive infants. The CD4/CD8 ratio was <1.0 in all but 6 infected children and in 14 of the 218 uninfected children (sensitivity 89%, specificity 93.5%).

Conclusion

Our findings suggest that the CD4/CD8 ratio may be used as a sensitive surrogate marker of HIV infection in an infant born to a HIV positive woman as CD4/CD8 counting facilities are more widely available than virological assays, in resource – poor settings. This would help in identifying infants for cotrimoxazole prophylaxis and/or ART.

Table 12: CD4+ & CD8+ T cell subsets in HIV-positive & HIV-negative infants

	CD4%	CD4 cell count (cells/mm^3)	CD8%	CD8 cell count (cells/mm^3)	CD4/CD8 ratio
HIV-positive (n=55)	19 ± 13*	1328 ± 1209*	48 ± 18*	3064 ± 2408*	0.51± 0.45*
HIV-negative (n=218)	43 ± 12	3188 ± 1500	22 ± 8	1663 ± 896	2.2 ± 1.2

*P value < 0.01

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

Ongoing studies

Antimycobacterial activity of actinomycetes isolated from less explored ecosystems

(Funded by Department of Science & Technology, New Delhi)

Background

The worldwide problem caused by TB and the lack of new drugs necessitate the search for novel drugs to control MDR-TB. Actinomycetes have long been considered as a source of high value metabolites especially antibiotics.

Aim

- To screen actinomycetes isolated from less explored ecosystems for antimycobacterial activity

Methods

Actinomycete strains isolated from less explored ecosystems like desert, marine, alkaline and forest soil samples were screened for antimycobacterial and antibacterial activity. The culture filtrates of fermented actinomycete strains were tested against *M. tuberculosis* H_{37}Rv, multi drug resistant isolate and a drug sensitive clinical isolate of *M. tuberculosis* by luciferase reporter phage (LRP) assay. Ethyl acetate extracts of fermentation broth and methanol extracts of

mycelium prepared from actinomycete strains were tested for antimycobacterial activity. Antibacterial activity was also studied by cross streak and disc diffusion methods against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*. Ethyl acetate and methanol extracts were tested against the bacterial strains by disc diffusion method.

Results

In LRP assay out of 53 actinomycetes culture filtrates tested, 17 showed antimycobacterial activity. Three out of 5 ethyl acetate extracts, 8 out of 30 methanol extracts and 1 out of 3 acetone extract showed antimycobacterial activity.

Conclusion

The ecosystems explored are potential sources for antagonistic actinomycetes as 17 out of 53 strains tested could inhibit *M. tuberculosis*.

The study is in progress.

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

Novel phage based assay for rapid detection of tubercle bacilli in pulmonary specimens for application in field conditions

Background

Rapid diagnosis of TB is vital for control and prevention of spread of the disease in the community. Assays aimed at field level applications should be simple, rapid, reliable and not require expensive instruments. Phages exhibit high level of host specificity, which make them ideal tools in specific diagnosis.

Aim

- To construct *lac*Z expressing mycobacteriophages and evaluate their role in rapid diagnosis of TB from pulmonary samples for application in field conditions

Methods

Established protocols were used for developing *lac*Z expressing mycobacteriophage constructs (blue phage constructs) from temperature sensitive mutant phAE159 of mycobacteriophage TM4. Standard molecular biology techniques were used for developing the required cosmids and plasmids. Diagnostic efficiency of one of the constructs was further evaluated using known cultures and sputum deposits.

Results

Conditionally replicating mycobacteriophage constructs harboring *lac*Z gene capable of detecting and reporting the viable tubercle bacilli in pulmonary specimens were developed. Using cultures, the sensitivity of detection of the phage construct was found to be 20 bacilli/ml. A preliminary evaluation of its diagnostic capability was done using 96 sputum samples processed by modified Petroff's method. The assay picked up 36 out of 45 conventional culture positives yielding a sensitivity of 80%. Among 51 culture negatives, 20 were assay negative yielding a specificity of 40% (table 13).

Table 13: Blue phage construct in comparison with conventional culture in diagnosis of TB

		LJ culture		Total
		Pos	Neg	
Blue Phage	Pos	36	31	67
	Neg	9	20	29
		45	51	96

All 9 culture positives that were missed by the assay were found to be high grade culture positives with a short turn around time indicating heavy load of viable bacteria in the sample calling for suitable modification of the assay so as to establish an ideal ratio of cells to phage. Among 31 culture negative-assay

positives, 4 were smear positive, 4 were from patients within 6 months of treatment, 6 within 1 year of treatment, 3 were follow up cases, 2 were established TB-HIV positive cases and 6 were single time samples from MDR suspects.

Conclusion

The construct shows promise based on the preliminary findings. The sensitivity and specificity of the assay can be greatly improved by optimizing the assay format and evaluating it against liquid based methods using larger number of samples. Such an attempt is fully warranted since the assay has the potential to completely eliminate the use of processing methods and expensive instruments.

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

Nevirapine and efavirenz concentrations in HIV-infected children treated with adult/pediatric antiretroviral drugs in India

Background

Antiretroviral drugs for pediatric use were made available by the NACO at the Government ART centres from November 2006. The most common pediatric formulation consists of NVP with 3TC and d4T in three different ratios, which are administered to children based on their body weight. Efavirenz is available as a single drug which is also given along with 3TC and d4T, usually to patients on concurrent anti-TB medications and those who cannot tolerate NVP. Underdosing is a major threat to the long term success of ART, and is of particular concern for children facing a life-time requirement for ART. It is therefore important to ensure that children receive adequate doses and plasma concentrations of antiretroviral drugs are maintained within the therapeutic range.

Aim

- To examine the influence of age, drug dose, type of formulation and nutritional status on blood levels of NVP and EFV in HIV-infected Indian children receiving treatment with generic adult or pediatric formulations

Methods

This was a multi-centric study conducted at four sites in three cities. HIV-infected children receiving treatment from the Government ART centres at the Government Rajaji Hospital, Madurai, B.J.Wadia Hospital, Mumbai, Government Hospital of Thoracic Medicine, Tambaram, Chennai and Kilpauk Medical College and Hospital, Chennai, and meeting the study criteria were recruited to the study. On the day of the study, blood samples (2 ml) were collected in heparinised vacutainers prior to drug intake (trough concentration) and at 2 hrs after administration of NVP and other antiretroviral drugs under supervision. In the case of EFV a single blood draw at 12 hrs after drug administration was performed.

Assessment of nutritional status

Nutritional status was graded and each child was assessed for underweight, wasting and stunting according to the WHO classification of malnutrition using Epi Info version 6.04d. Z scores of height-for-age, weight-for-age and weight-for-height were used to measure stunting, underweight and wasting respectively.

Drug estimations

Plasma concentrations of NVP and EFV were determined by validated HPLC assays with UV detection. A total of 96 HIV-infected children have been recruited to the study. Among them, 80 and 16 respectively were receiving NVP and EFV-based ART regimen. The study data is being analysed.

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

Monitoring plasma nevirapine and efavirenz in HIV-TB patients undergoing anti-TB and antiretroviral treatment

Background

Although it is known that RMP significantly reduces the bioavailability of EFV and NVP, the clinical significance of this reduction is unclear. In an ongoing controlled clinical trial at the Centre, two different antiretroviral regimens along with RMP containing ATT are being evaluated in patients with HIV-1 and TB.

Aim

- To study the trough levels of EFV and NVP while receiving ART (with and without ATT) and correlate with treatment outcome (viral load and CD4 cell count measurements) and NVP/EFV resistance pattern

Methods

The study is being carried out in patients who are getting recruited into the ongoing controlled clinical trial. The trough levels of NVP and EFV is being studied at 4 time points, that is, at months 1, 4, 6 and 12 after start of ART (while receiving ART & ATT and only ART). At these time points, a sample of blood (3 ml) in heparinised vacutainer is collected before the patients have taken their drugs. This will represent trough concentrations of NVP or EFV. Plasma NVP and EFV are estimated by HPLC according to validated methods.

So far, 64 and 94 patients receiving NVP and EFV respectively have been recruited into this study.

The study is in progress.

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

ICMR-Biomedical Informatics Centre

The Bioinformatics facility at TRC has been widely used by many research scholars and students from TRC as well as other institutes. Fifteen students have carried out their academic project at TRC during this year for degree programs such as M.Sc., MCA, B. Tech., PGD in Bioinformatics and B.Sc., from various academic institutions. Further, the Centre has conducted a workshop on "Perspectives of Biomedical Informatics Centre" which was attended by 25 participants including 18 faculties from different Medical Colleges in and around Chennai. The centre has contributed to five research projects of TRC.

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

Completed studies

Potential drug targets against drug resistant tuberculosis

Background

M. tuberculosis has evolved into a drug resistant species. *M. tuberculosis* resistant to INH and RMP is referred to as MDR-TB, while *M. tuberculosis* resistant to INH, RMP, one of the quinolones and one of injectable drugs is called extensively drug-resistant TB (XDR-TB). XDR-TB does not respond to most of the anti-TB drugs and MDR-TB has the potential to evolve into XDR-TB. In some parts of the world the incidence of MDR-TB is as high as 14% and XDR-TB cases have been confirmed in all regions of the world. The lethal combination of drug-resistant TB and HIV infection is a growing problem that presents serious challenges for effective TB control. Hence there is an urgent need to develop alternative drugs to combat TB.

To develop drugs for any disease, it is first important to identify candidate genes which could serve as potential drug targets. In this study a simple but significant strategy has been employed to identify such genes in *M. tuberculosis. M. tuberculosis* has 3989 known genes and 112 worked out metabolic pathways. The targets of all currently available anti-TB drugs are enzymes involved in 12 of

these pathways. We hypothesize that any other enzyme involved in these pathways could also be potential drug targets.

Aim

- To identify novel drug targets from the metabolic pathways involving drug resistant genes

Methods

All proteins (372) involved in the 12 known drug resistance linked metabolic pathways were downloaded from the SWISPROT database. Among the 372 proteins, 38 were found to be involved in more than one pathway, bringing down the number of unique proteins to 334. Of these, 18 proteins are targets for currently used anti-TB drugs, reducing the number to 316. The 316 proteins were compared with the human proteome using BLASTP in order to identify and exclude mycobacterial proteins with human homologs (e value of 0.005). This process excluded 221 proteins, leaving out 95. Since these proteins are involved in proven drug targeted metabolic pathways of *M. tuberculosis* and have no significant homologs in humans, they possess the potential to qualify as drug targets. Since drug development is a very expensive process, narrowing down the number of candidates using stringent criteria would be highly beneficial. We have therefore applied additional filters, viz., essentiality (for survival of mycobacteria), virulence and drug regulation (genes regulated by anti-mycobacterial drugs), to identify drug targets with high potential.

Results & Conclusions

Among the 95 proteins identified by us, 63 proteins were already identified as drug targets by various groups while 32 proteins were predicted in this study for the first time as drug targets. Further, of the 95 probable drug targets, 34 were found to be essential for *M. tuberculosis* (Database of Essential Genes), 3 were involved in the pathogenesis/virulence of *M. tuberculosis* (Virulence Factor Database) and 3 were found to be regulated by the action of capreomycin (a second-line anti-TB drug) (microarray data), thereby adding significance to their value as potential drug targets for *M. tuberculosis* (tables 14 & 15).

Table 14: Potentials drug targets from metabolic pathways linked with drug resistance also regulated by anti-TB drugs

Sl. No.	Rv No.	Swissprot ID	Description	Metabolic Pathway	E value	PDB ID
1	Rv0651	P66044	50S ribosomal protein L10	Ribosome metabolism mtu03010	5.8	NA
2	Rv1015c	P66121	50S ribosomal protein L25	Ribosome metabolism mtu03010	3.6	1DFU
3	Rv1547	P63977	DNA polymerase III subunit alpha	Purine metabolism mtu00230	0.35	NA

Table 15: Potential drug targets from metabolic pathways linked with drug resistance also involved in virulence

Sl. No.	Rv No.	Swissprot ID	Description	Metabolic Pathway	E value	PDB ID
1	Rv0642c	Q79FX8	Methoxy mycolic acid synthase 4 (MMAA4)	Tryptophan metabolism mtu00380	0.058	NA
2	Rv2959c	Q50457	Rhamnosyl O-methyl transferase	Tryptophan metabolism mtu00380	0.097	NA
3	Rv3601c	P65660	Aspartate 1-decarboxylase	Alanine and aspartate metabolism mtu00252	5.3	2C45

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

Database for drug resistant mutations in *M. tuberculosis*

Background

The emergence of drug resistant TB is of great concern since there is no cure for XDR-TB and there is growing concern that it may spread around the world, stressing the need for additional control measures such as new diagnostics and

better drugs for treatment. The primary mechanism of drug resistance in *M. tuberculosis* is the accumulation of mutations in genes coding for drug targets or drug-converting enzymes. Therefore it is necessary to identify all mutations which cause resistance to anti-TB drugs. Molecular mutations causing resistance for most of the currently used anti-TB drugs have been identified. However, this information has not been consolidated and stored systematically for clinical applications as has been done for HIV.

Aim

- To consolidate all known mutations causing resistance against anti-TB drugs

Methods

DNA and protein sequences of *M. tuberculosis* H37Rv from GenBank (NC_000962) were used as reference. All DNA sequences with drug resistance mutations deposited in GenBank were obtained using BLASTN. Their corresponding proteins sequences were also retrieved. All hits were compared individually with the reference sequence to identify the location of the mutations.

Result & Conclusions

One hundred and fifty nine sequences reported to contain mutations conferring resistance to anti-TB drugs were retrieved from GenBank. Among *katG*, *inhA, rpoB, pncA, embA, embB, gyrA,* and *rpsL*, *katG* was found to have highest (66) number of drug resistant sequences. Frequently occurring mutations in individual genes were also identified. The results are stored as a local database at the Biomedical Informatics Centre, TRC. This database would serve as a platform to carry out structural bioinformatics studies on drug resistant TB and pave the way to computer aided drug designing.

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

In silico modeling of mutant pyrazinamidase genes of *M. tuberculosis* and docking with pyrazinamide

Background

Pyrazinamidase (PZAase) plays a key role in activating the prodrug PZA, an important drug in the anti-TB therapy. Mutations in *pncA* gene coding for PZAase are a major mechanism of PZA resistance in *M. tuberculosis.* In this study, mutant PZAases were modeled using Discovery Studio.

Aims

- To predict the three dimensional structure of mutant PZAase
- To study the conformational changes due to the various mutations
- To predict the binding modes of PZA to wild type and mutated models of PZAase from docking calculations

Results & Conclusions

The template chosen was PZAase from *Pyrococcus hirokoshi* (1im5), which had 37% identity with the target protein. It comprises of 7 helices and 6 β strands. The amino acids identified at the active site were Lys96, Asp49, Asp8, Cys138, Trp68, Phe13, Ala134, and Thr135. Models for mutated PZAase at Cys138 to tyrosine and serine were predicted to study conformational changes leading to drug resistance. This study would help in better understanding of the mechanisms of drug resistance. In addition, identification of mutations in the active site of the target protein would give an insight on the drug-target interactions, leading to the rational design of more efficacious drugs that may shorten therapy.

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

Tape measure protein having MT3 motif facilitates phage entry into stationary phase cells of *M. tuberculosis*

Background

Tape measure protein (TMP) having MT3 motif in mycobacteriophage TM4 genome has been reported to enable the phage infection of *M. smegmatis* during stationary phase.

Aims

- To identify MT3 motifs in the genome of mycobacteriophages
- To corroborate the presence or absence of MT3 motif in TMP protein of these phages with the ability to infect stationary phase cells of *M. tuberculosis*

Results & Conclusions

TMP of eight additional mycobacteriophages were analyzed using *in silico* methods for the presence of MT3 motifs. Six were found to possess MT3 motifs. To validate the hypothesis that the absence of MT3 motif in Che12 and D29 makes them incapable of infecting stationary phase cells of *M. tuberculosis,* laboratory experiments were performed to test the performance of respective LRP constructs developed from the parental phages Che12, D29 and TM4.

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

Three dimensional modeling of Rv2989 and identification of promoter region for Rv2991

Background

Isocitrate lyase regulatory protein (IclR) family of transcription factors are widely distributed in bacteria and their products are involved in various functions like glyoxylate shunt, multi-drug resistance, quorum-sensing signals, sporulation, control of efflux pumps, etc. The IclR family is named after the well characterized IclR protein of *E. coli*, which controls the glyoxylate bypass. IclR family is defined based on the sequence similarities in the region between residues 151 and 229 of the *E. coli*-IclR primary sequence. *M. tuberculosis* H37Rv has 3 IclR types of transcriptional regulators (TRs): Rv1719, Rv1773c and Rv2989. These regulators contain possible helix turn helix (HTH) motifs and share similar amino acid sequences. However, the role of the IclR type of TRs in *M.tuberculosis* has not been addressed so far. The putative IclR type transcriptional regulator Rv2989 is similar to SRPS efflux pump regulator from *Pseudomonas putida* (28.35% identity in 247 amino acids overlap; http://genolist.pasteur.fr/TubercuList/).

Characterization of this putative TR might provide a clue to its function in *M. tuberculosis*.

Aims

- To predict the three dimensional structure of Rv2989 and DNA binding region
- To predict the promoter region of Rv2991

Results & Conclusions

We identified a HTH motif in the IclR domain of Rv2989 using *in silico* approaches. Further, we predicted the three dimensional structure of Rv2989 protein and identified its possible DNA-binding sequence. We also identified the conserved residues by performing multiple sequence alignment of Rv2989 with other known proteins having IclR and HTH domain. We have also predicted the promoter region of Rv2991.

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

Synonymous codon usage analysis of 32 mycobacteriophage genomes

Background

Modification of the codons of the luciferase gene with respect to the optimal codons of the phage and the host should lead to better expression of the same, improving the sensitivity of mycobacterial detection by the LRP assay. The present venture to study and understand codon usage patterns of all the mycobacteriophages so far sequenced is thus aimed at optimizing the LRP assay.

Aim

- To study and compare the synonymous codon usage of 32 completely sequenced mycobacteriophage genomes

Results & conclusions

Synonymous codon usage of protein coding genes of 32 completely sequenced mycobacteriophage genomes was studied using multivariate statistical analysis. One of the major factors influencing codon usage was identified to be compositional bias. Codons ending with either C or G were preferred in highly

expressed genes among which C ending codons were highly preferred over G ending codons. Translational selection was also identified to play a role in shaping the codon usage operative at the level of translational accuracy. High level of heterogeneity was seen among and between the genomes. The length of genes was also identified to influence codon usage in 11 of the 32 phage genomes. *Mycobacteriophage cooper* was identified as the most highly biased genome with better translational efficiency.

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

Ongoing studies

Database for drug resistant diseases

The discovery and development of antibiotics, which were first employed in medical practice in the 1940s, stands as one of the major accomplishments of medicine. However, increasing antimicrobial drug resistance is now threatening these medical advances. Deaths from acute respiratory infections, diarrheal diseases, measles, AIDS, malaria and TB account for more than 85% of the mortality from infections worldwide. Resistance to first-line drugs in pathogens causing these diseases ranges from 0 to almost 100%. In some instances resistance to second and third line agents is seriously compromising treatment outcome. Highly resistant organisms are virtually untreatable in immunocompromised patients. Lessons from TB, malaria, HIV, MRSA (methicillin resistant *Staphylococcus aureus*) and innumerable other drug-resistant pathogens have taught us that drug resistance, once established, is almost certain to escalate.

Aim

- To develop a biological database for drug resistant diseases

This database would contain information about available drugs, drug targets, genes involved in drug resistance, metabolic pathways involving these genes and potential drug targets.

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

Identification of novel substrates of *M. tuberculosis* PknE

Background

To challenge the global threat posed by *M. tuberculosis*, it is important to have a better understanding of its physiology. PknE and other serine/threonine protein kinases are involved in signaling network and in a range of control mechanisms. Identifying the downstream targets for these kinases will help to gain detailed molecular understanding of the full repertoire of cellular functions that can be modulated by extracellular signals.

Aim

- To identify novel substrates of the PknE protein of *M. tuberculosis*

Results

We have predicted the possible substrates of PknE using bioinformatics tools. These need to be further short listed.

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

Mycobacteriophage genome database - *in silico* annotation of genes and proteins

Background

Complete genome sequences are available in the public domain for 45 mycobacteriophages. We made an attempt to develop a fully annotated database of these mycobacteriophages.

Aims

- To predict the function and to characterize the genes and protein products of mycobacteriophages using various bioinformatics tools
- To perform a comparative analysis of mycobacteriophage genomes with mycobacterial and other bacterial genomes
- To develop a backend database for genome and gene information using MySQL
- To develop a PHP-based graphical user interface for querying and displaying genome and gene information of mycobacteriophages

Mycobacteriophages are viruses that are capable of infecting mycobacteria. Studies on these phages led to the understanding of mycobacterial genetic systems providing insights into viral diversity and the evolutionary mechanisms that generated them. Because of their ability to specifically infect and replicate in mycobacterial cells, mycobacteriophages have the potential to become useful tools in the diagnosis of TB. The genomes of 32 mycobacetriophages are completely sequenced and are available in online databases. However, these phage genomes have not been well characterized. Understanding the function of the genes and their protein products are important for exploring the life cycle, host-phage interaction, etc. of the organism. To fulfill these requirements, we made an attempt to develop a comprehensive database for mycobacteriophage genomes. The database provides comprehensive information on genome sequence, genes and proteins as well as their properties that may lead to novel predictions and functional inference for those proteins that were previously uncharacterized.

Results

Currently we have annotated 28 genomes using various *in silico* tools and have uploaded them in our local database. The remaining 17 genomes are being annotated.

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

Studies on the binding modes of isoniazid to wild type and mutant forms of *katG*

Background

Emergence of drug resistance has been linked to the occurrence of mutations either in the bacterial drug target proteins and/or their associated proteins. Resistance to INH, the potent inhibitor of *M. tuberculosis katG* protein, is mainly related to mutations at residues 315 and 138 of the enzyme.

Aims

- To model the mutant structures of *katG*
- To study the interaction energy between *katG* and inhibitor molecules

Results

3D models were predicted for five mutant forms of *katG,* using the wild type *katG* as the template. We have also studied the interaction of INH with these mutants. Further work in this line is in progress.

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

BASIC RESEARCH

Completed studies

Characterization of HIV-1 isolates and pattern of drug resistance from patients initiating ART

Background

Antiretroviral combination therapy is a major advance in the treatment of HIV infection. Two classes of antiretroviral drugs (ARVs) are now available to treat HIV-1 infection through the NACO-ART program in India (nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs)) while protease inhibitors have been made available for 2nd line indications. The emergence of resistance to these ARVs is an important cause of treatment failure. The viral mutants exhibit genotypic mutations in reverse transcriptase (RT), protease (prot) & envelope (env) genes. These mutations have been well characterized in subtype B strains, because this subtype is predominant in the western world where ARVs have been extensively used. In developing countries like India where infections with HIV-1 subtype C is predominant and access to ARVs has been quite recent, it could be possible that the pattern of drug resistant mutations (DRMs) are different from that seen in the subtype B isolates. This study will help us to identify mutant genotypes in this population and provide the opportunity for early intervention and effective management.

Aim

- To delineate the DRMs in ART-naïve HIV-positive patients in south India

Methods

Pattern of polymorphism and potential DRMs were evaluated in HIV-1 isolates from 108 HIV-1/TB co-infected individuals naïve to ART, attending the outpatient clinics of the TRC. The samples were subjected to genotyping of HIV-1 RT and prot genes.

Results & Conclusion

108 HIV-infected ART-naïve patients with TB were screened for DRMs in the RT and prot genes of the HIV by genotypic method. Of these patients, successful

amplification was achieved in 106 patients at baseline (105 for RT and 101 for prot genes). Significant polymorphisms were observed in both RT gene and prot genes in all naïve patients, while 1 patient each had naturally occurring resistance mutations to NRTI (M184V), NNRTI (Y181C) and prot (L90M). 19 patients (having viral load >5000 copies/ml plasma) failed therapy at the 6th or 12th month of treatment. All patients who failed treatment had resistant mutations to at least one of the RT inhibitors while none of them had mutations in the prot gene. The pattern of DRM present in the patients failing treatment is presented in the table 16. The commonest mutations observed were M184V (3TC), Y181C (NVP/EFV), and G190A and L74V (NRTIs). The pattern of mutations described on failure of 1st line ART will be useful in the design of second-line regimens for HIV-infected patients in India.

Table 16: Pattern of drug resistance mutations in patients failing ART

	Mutations Associated with Resistance to NRTI and NNRTI (n=19)																			
CODON	**K65**	**L74**				**K101**		**K103**	**V106**		**V108**		**Y181**		**M184**				**G190**	**K219**	
AA CHANGE	KR	V	L	LV	I	E	KE	N	M	MV	I	IL	C	VY	V	IM	IMV	IV	A	E	N
NO OF SAMPLES	1	5	1	1	1	2	1	3	4	1	3	1	10	1	11	1	1	1	5	1	1

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

Cytokine gene polymorphisms and cytokine levels in pulmonary TB

Background

Th1 and Th2 cytokines play an important role in the immune response against TB and alteration in their levels might contribute to the outcome of the infection. Single nucleotide polymorphisms in the cytokine genes may influence the cytokine levels in pulmonary TB which may be associated with susceptibility or resistance to TB.

Aim

- To find out the influence of variant genotypes of cytokine genes on cytokine levels and their association with pulmonary TB

Methods

The study subjects included 166 pulmonary TB patients (PTB) and 188 normal healthy subjects (NHS). Genotyping of IFN-γ (+874 and +5644), IL-2 (-330 and +160), IL-4 variable number tandem repeat, IL-6 (-174), IL-10 (-1082 and -819) and IL-12B 3'untranslated region +1188 polymorphisms were done using polymerase chain reaction-allele specific primer (PCR-ASP) and PCR-RFLP methods. To study the influence of cytokine gene polymorphisms on cytokine levels, phytohaemagglutinin and culture filtrate antigen (CFA) of *M. tuberculosis* induced cytokine levels were measured by ELISA from 72 hr old peripheral blood mononuclear cell culture supernatants.

Results

Significantly increased frequency of G allele [Odds ratio (OR) 1.46, 95% confidence interval (CI) 1.07-1.98] and decreased frequency of T allele (OR 0.69, 95% CI 0.50-0.94) of IL-2 -330 (T/G) polymorphism was observed among PTB patients compared to NHS (p=0.017). The TT genotype of IL-2 -330 polymorphism was significantly under represented in patients as compared to NHS (p = 0.024, OR 0.53, 95% CI 0.31-0.92) (table 17). Genotype frequencies of other polymorphisms were not significantly different between NHS and PTB. IL-12p40 levels were significantly lower in NHS with AA genotype than in NHS with AC genotypes ($p<0.05$). In PTB patients, spontaneous IL-12p40 levels were significantly higher among patients with CC genotype compared to patients with other genotypes ($p<0.01$). CFA induced IL-12p40 levels were significantly higher in patients with CC genotype compared to patients with AC genotype ($p<0.05$) (Fig.4). No significant differences in the levels of other cytokines between the variant genotypes of various cytokine gene polymorphisms were observed between NHS and PTB patients.

Conclusions

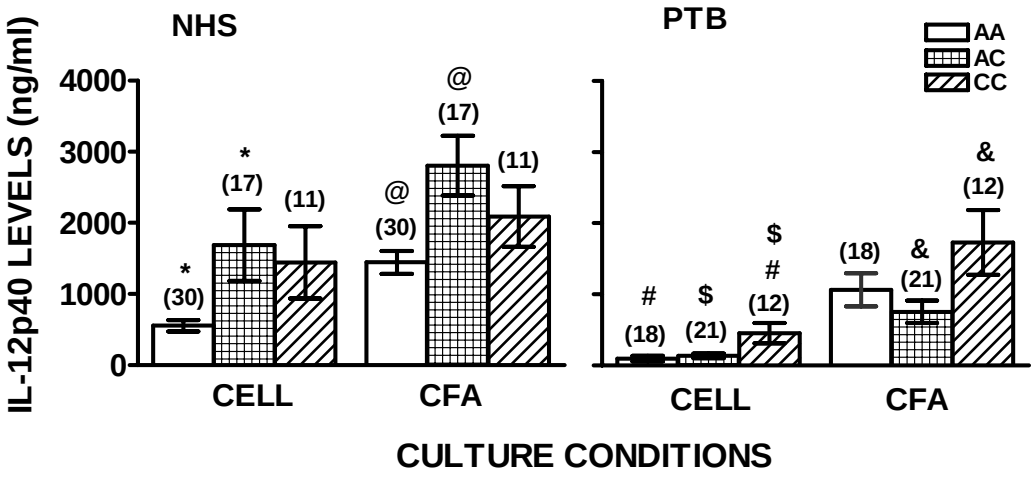
The present study suggests that the TT genotype of IL-2 -330 polymorphism may be associated with protection to PTB. Further, +1188 polymorphism of IL-12B gene either, alone or in combination with closely linked genes may regulate IL-12p40 production and may play a major role on acquired immunity to TB.

Table 17: Allele and genotype frequencies of IL-2 -330 gene polymorphism in NHS and PTB patients

IL-2 Gene variants	NHS n = 187	PTB patients N = 164
-330 (T/G) Alleles		
T	0.529	0.436
G	0.471	0.564
Genotypes		
TT	27.8 *	17.1 *
TG	50.3	53.0
GG	21.9	29.9

n = subjects studied; Genotype given as percent frequencies.
*p = 0.024; OR 0.53, 95% CI 0.31- 0.92

Fig.4:



Influence of IL-12B gene 3' UTR +1188 polymorphism on spontaneous cell and CFA of *M. tuberculosis* induced IL-12 production in NHS and PTB patients. The values are expresses as mean. The vertical bars represent SE. Numbers in the parentheses represent individuals studied. In NHS, AA versus AC * $p<0.05$, @ $p<0.01$; In PTB, CC versus AC and AA # $p<0.01$, $ $p<0.01$ and CC versus AC & $p<0.05$.

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

Human Leucocyte Antigen -DPB1 (HLA -DPB1) and vitamin D receptor gene polymorphism studies in HIV and HIV-TB patients

Background

Our earlier studies revealed the association of HLA-A11 with resistance and HLA-B40 and -DR2 with susceptibility to HIV and HIV-TB. Other polymorphisms in the HLA and non-HLA region might also influence susceptibility or resistance to HIV-1 infection and development of TB in HIV-1 infected patients.

Aim

- To find out whether polymorphisms in HLA -DPB1 and Vitamin D receptor (VDR) genes are associated with susceptibility or resistance to HIV and HIV-TB

Methods

The study subjects included 131 HIV-positive TB-negative patients (HIV+TB-), 82 HIV-positive pulmonary TB-positive patients (HIV+PTB+), 107 HIV-negative pulmonary TB positive patients (HIV-PTB+) and 146 healthy controls. HLA -DPB1 typing was done by PCR with locus specific primers followed by hybridization with biotinylated allele specific oligonucleotide probes (ASOP) and detection by chemiluminescence method. VDR gene polymorphisms in the 5' regulatory region (Cdx2 and A-1012G), coding region (*Fok*I) and 3' untranslated (UTR) region (*Bsm*I, *Apa*I and *Taq*I) were studied using PCR-ASP and PCR-RFLP methods.

Results

HLA-DPB1*1501 was significantly under represented in HIV-PTB+ patients (p=0.002, P_c=0.034, OR 0.07, 95% CI 0.00-0.49) and HIV+PTB+ patients (p=0.036, OR 0.13, 95% CI 0.00-0.92) compared to healthy controls (table 18).

Among the 5' regulatory and coding region polymorphisms of VDR gene, significantly increased frequency of GA genotype of Cdx-2 was observed in HIV+TB- group compared to controls (p=0.012, Odds ratio (OR) 1.89 95% Confidence interval (CI) 1.14-3.15). In the 3' UTR genotypes, a trend towards decreased frequency of bb genotype of *Bsm*I in HIV+TB- (p = 0.052, OR 0.56

95% CI 0.31-1.00) and increased frequencies of AA genotype of *Apa*I in HIV+PTB+ patients (p=0.056, OR 1.80 95% CI 0.98-3.30) and tt genotype of *Taq*I in HIV+PTB+ patients (p=0.05, OR 2.32 95% CI 0.99-5.46) were observed compared to controls. Haplotype analysis revealed significantly increased frequencies of 3'UTR haplotype B-A-t in HIV+PTB+ group (Pc = 0.030, OR 1.75 95% CI 1.14-2.66) and decreased frequencies of b-A-T haplotype in HIV+TB- (p = 0.031 OR 0.48 95% CI 0.25-0.89) and HIV+PTB+ groups (Pc = 0.04, OR 0.47 95% CI 0.23-0.89) compared to controls (table 19).

Conclusions

The results suggest that HLA -DPB1*1501 may be associated with protection against pulmonary TB development both in HIV-positive and negative subjects.

Results on VDR gene polymorphisms suggest that VDR gene 3'UTR haplotype b-A-T may be associated with protection against HIV infection while B-A-t haplotype might be associated with susceptibility to development of TB in HIV-1 infected patients.

Table 18: Percent frequencies of significant HLA -DPB1 alleles in patient groups & healthy controls

HLA -DPB1 alleles	Healthy controls n=112	HIV+PTB- n=115	HIV+PTB+ n=59	HIV-PTB+ n=110
*0201	23.2[¶]	28.7	40.7[¶]	27.3
*1501	11.6[#,§]	8.7	1.7[§]	0.9[#]

n= number of individuals studied;
[¶] HIV+PTB+ vs Healty controls, p=0.027, OR 2.27, 95% CI 1.08-4.72.
[#] HIV-PTB+ vs Healthy controls, p=0.002, OR 0.07, 95% CI 0.00-0.49.
[§] HIV+PTB+ vs Healthy controls, p=0.036, OR 0.13, 95% CI 0.00-0.92.

Table 19: Percent frequencies of selected genotype & haplotypes of VDR gene polymorphisms in HIV-1 infected patients & healthy controls

Genotypes/ haplotypes	Healthy Controls n=146	HIV+ TB- n=131	HIV+PTB+ n=82	HIV-PTB+ n=108
Genotypes				
Cdx2 GA	41.1	56.9	48.1	61.2
*Bsm*I bb	30.8	19.9	19.7	26.2
*Apa*I AA	30.1	26.7	43.8	40.0
*Taq*I tt	9.6	9.3	19.8	16.5
Haplotypes				
B-A-t	29.0	36.1	41.1	34.6
b-A-T	13.3	6.8	8.0	10.1

n = individuals studied; Genotype and haplotype frequencies are given in percentage

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

Levels of complement in TB

It has been shown that there is hypercatabolism of complement system, increase in circulating immune complex levels along with a reduction in erthyrocyte complement receptor-1 (CR-1) and complement-mediated immune complex solubilization. Further, there is an increase in the HH phenotype of CR-1. It was also found that the addition of complement to peripheral blood leucocytes resulted in a reversal of the cytokine imbalance normally observed in patients with pulmonary TB.

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

Activation of complement system by genetically modified mycobacteria

It was shown that devR mutant of *M.tuberculosis* was able to activate C3 less, although assembly of C4 or factor B was nomal in comparison with the wild type *M. tuberculosis* bacilli. Studies with mutants lacking tyrosine phosphatase genes also indicated that these enzymes probably modulate the production of pro inflammatory cytokines.

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

The role of antibody in the interaction between *M. tuberculosis* and macrophages through the complement system

It was found that the addition of antibodies augmented complement-mediated phagocytosis as well as apoptosis. There was a downregulation of complement-receptor expression on both CD4 and CD8 cells when *M.tuberculosis* opsonized with antibody and complement interacted with them. Further, there was a reduction of TNF-α and an increase in IL-6 levels.

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

Molecular characterization of *cis* and *trans* acting elements of acetamidase operon of *M. smegmatis*

Background

The highly inducible enzyme, acetamidase of *M.smegmatis* enables the organism to utilize several amide compounds as sole carbon source including acetamide and formamide. This enzyme is expressed in basal level in non-induced conditions and 100 fold induced in the presence of an inducer like acetamide and can be visualized as 47kDa band in SDS-PAGE. It is part of an operon, the acetamidase operon of *M. smegmatis* which has other four predicted open reading frames (ORFs), which are supposed to be involved in the regulation of this operon. Earlier we reported the cloning of the predicted four ORFs (AmiC, A, D and AmiS) upstream to the acetamidase enzyme and expression and purification of two of these using *E .coli* expression system. Here

we report the characterization of a regulatory protein, AmiA which was predicted to be a MarR family of repressor with Helix-turn-helix motif.

Objective

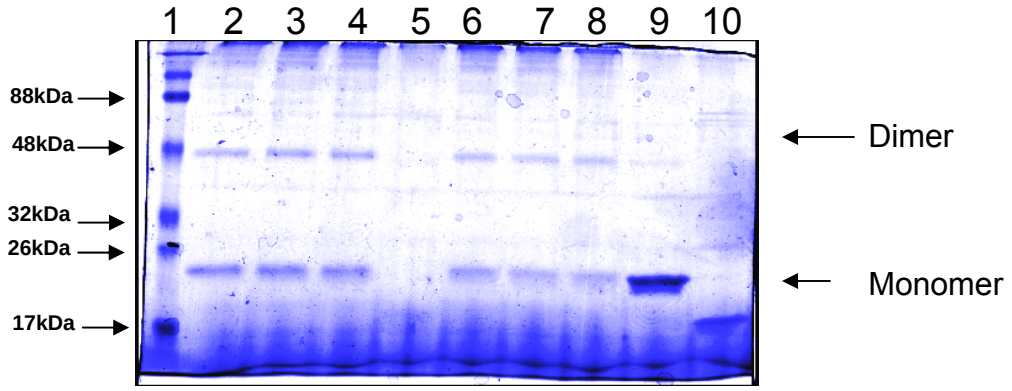
- To characterize the promoter of acetamidase operon

Results

Using glutaraldehyde cross linking of proteins it was found that the AmiA protein forms a homodimer (Fig.5). Previous studies in our lab signified the possibility of upstream acting elements in the operon. To show the interaction between the AmiA which is a predicted repressor, with these elements, electrophoretic mobility shift assay (EMSA) was performed using the PCR generated and γ-P^{32}-dCTP labeled DNA fragments. Initially binding ability of this protein within the operon was screened using PCR generated fragments of 300bp length. From this it was established that fragments **L** and **M** which comprises of 1654 – 1975bp and 1776 – 2104bp of the operon, specifically bind with the protein (Fig.6A). The length of this fragment was further minimized to ~ 150 - 200bp and found that the fragment comprising 1776 – 1975 (Fragment **ML**) of the operon specifically binds with the protein (Fig.6B). Previous studies from our lab showed that this region has promoter activity. It was further confirmed that AmiA acts as a repressor and binds in the operator region of acetamidase operon. Further work would be carried out using footprinting assay to exactly pinpoint the bases involved in binding.

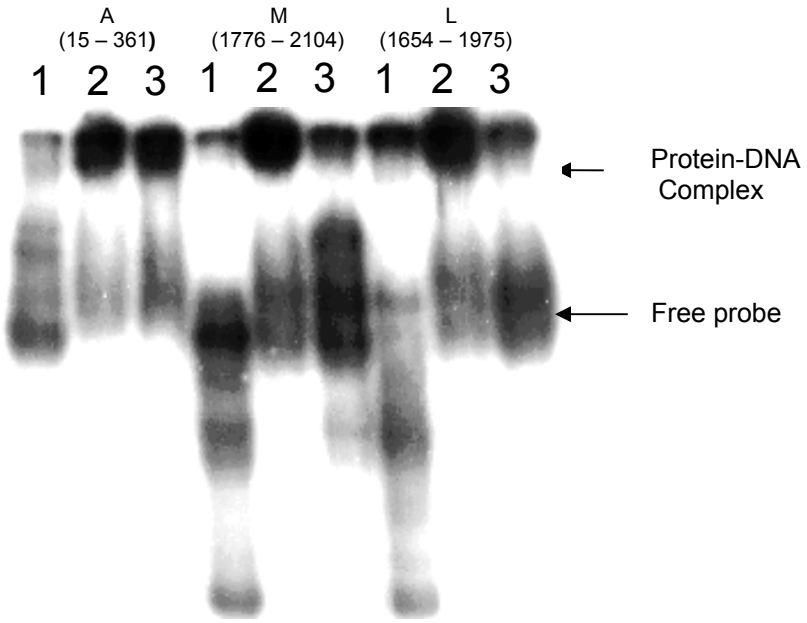
Over expression of AmiC, AmiA and AmiD was attempted using *hsp* promoter driven vector pMV261. Over expression of AmiA in parallel with the wild type operon in *M .smegmatis* greatly influenced the expression of acetamidase. As observed by SDS-PAGE, cells harboring no extra copy of AmiA expressed high level of acetamidase and this increased with increasing concentration of acetamide. However, the cells harboring extra copy of AmiA expressed significantly lower levels of acetamidase than the controls as observed in the protein band intensity at 47kDa (Fig.7). The acetamidase was negatively regulated by AmiA, whose over expression abolished the inducibility by acetamide.

Fig.5: Glutaraldehyde cross linking of AmiA



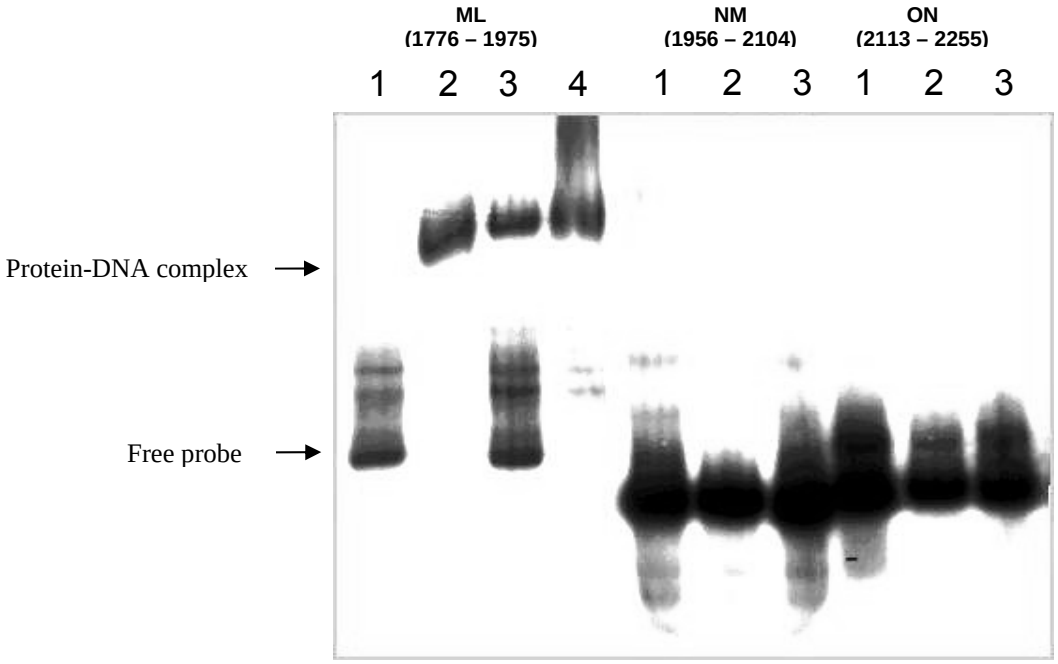
Lane 1: Molecular weight marker, Lanes 2-4: AmiA cross linked with different concentration of glutraldehyde, Lane 5 empty, Lanes 6-8 cross linking of AmiA and AmiD, Lane 9 and 10 has control AmiA and AmiD respectively

Fig.6A: EMSA with ~300bp PCR fragments



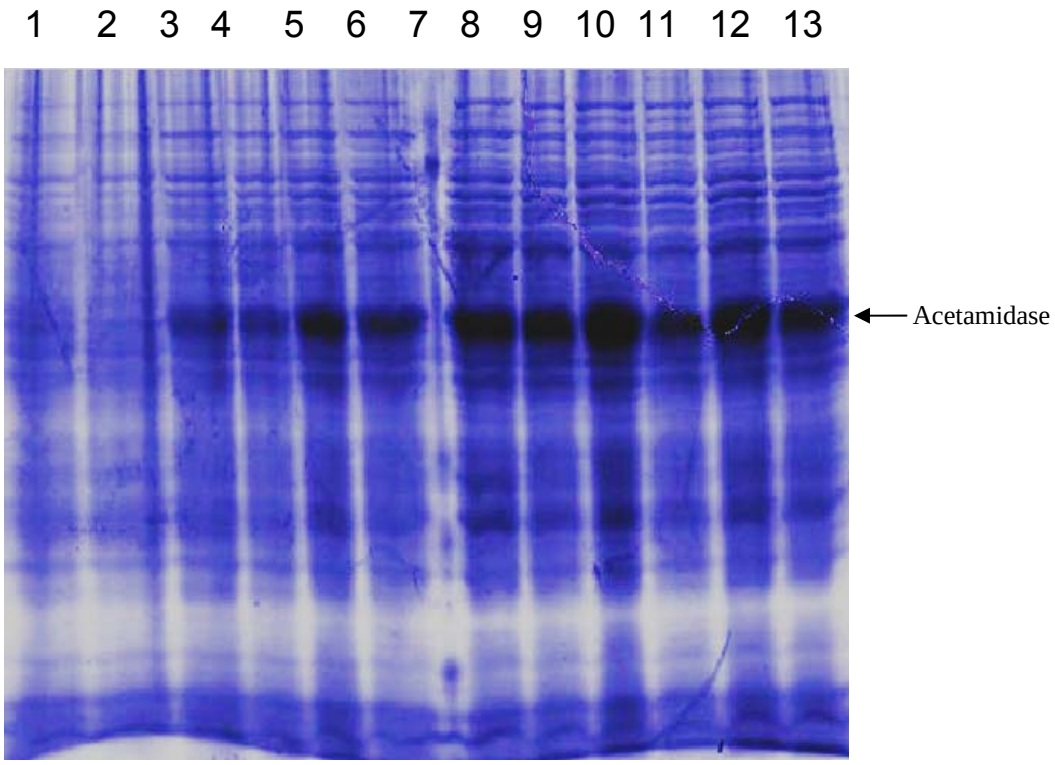
Lane 1: Free probe, Lane 2: 1X, Lane 3: 100X cold chase

Fig.6B: EMSA with ~150 - 200bp PCR fragments



Lane 1: Free probe, Lane 2: 1X, Lane 3: 100X cold chase and Lane 4: nonspecific cold chase

Fig.7: Over expression of AmiA and its effect on the expression of acetamidase



Lane 1: MC² with 0mM acetamide, Lane 2: ORF1 with 0mM acetamide, Lane 3: MC² with 5mM acetamide, Lane 4: ORF1 with 5mM acetamide, Lane 5: MC² with 15mM acetamide, Lane 6: ORF1 with15mM acetamide, Lane 7: Marker, Lane 8:MC² with 25mM acetamide, Lane 9: ORF1 with 25mM acetamide, Lane 10: MC² with 35mM acetamide, Lane 11: ORF1 with 35mM acetamide, Lane 12: MC² with 50mM acetamide Lane 13: ORF1 with 50mM acetamide.

Future studies

We are standardizing footprinting analysis to find the exact DNA binding site for AmiA protein on the operon and future experiments are designed to find the protein – protein interaction among the three regulatory proteins of acetamidase operon namely, AmiC, AmiA and AmiD.

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

MEK activation by different strains of *M. tuberculosis*

Background

Mycobacteria successfully parasitize the host macrophages by inhibiting various host-cell responses. The mycobacteria employ several strategies to subvert host-cell signaling; identification of key molecules involved in signaling, might serve as potential targets for new antimycobacterial therapies. However, the different mechanisms adopted by pathogenic nonpathogenic mycobacteria in subverting the signalling machinery of host cells is not clear. Any study directed towards delineating the mechanisms that are involved in mycobacterial modulation of macrophage survival and death will provide a useful insight in understanding host–mycobacteria interactions. There have been several reports about the role played by signaling pathways in modulating the immune responses. Mitogen activated protein kinases (MAPK) signaling is one of the several pathways which plays a key role in mycobacterial pathogenesis.

Aim

- To study whether the genotype of the *M. tuberculosis* strains influence the cell signaling mechanism

Method

Epidemiologically well characterized strains were chosen and activation kinetics of MEK in THP-1 cells infected with different *M. tuberculosis* strains was studied. The following *M. tuberculosis* strains were used in the study:

- **H37Rv** – virulent laboratory reference strain *M. tuberculosis*
- **H37Ra** – avirulent laboratory reference strain *M. tuberculosis*
- **1338** – clinical isolate obtained during the Model Dots study conducted at the Bacillus Calmette Guerin (BCG) trial area of Tiruvallur District. This strain has a single copy of the IS6110 insertion sequence and was highly prevalent in the community based on previous RFLP studies. In animal studies, using guinea pig model, this strain had exhibited low virulence.
- **2567 or S7** – clinical isolate obtained during the Model DOTS study conducted at the BCG trial area of Tiruvallur District. This strain has a

single copy of the IS6110 insertion sequence and was prevalent in the community, based on previous RFLP studies. It induced the proliferation of predominantly Th2-type cells secreting IL-4, which in turn suppressed Th1 response in healthy subjects.

- **Beijing strain** –Among the isolates screened by spoligotyping from Model DOTS area, a strain with Beijing spoligotype was chosen which was found to be resistant to INH. The prevalence of the Beijing strain in Tiruvallur district is very low (3%).

THP-1 cells were seeded in 24-well tissue culture plates at a density of 0.5×10^6 cells per well and cultured with medium alone (control), or infected with *M. tuberculosis* strains at a multiplicity of infection bacteria: monocyte ratio of 10:1 (or 1 µg/ml Lipopolysaccharide (LPS) derived from *Escherichia coli*, serotype 055:B5 as the positive control) for various periods (15, 30, 45, 60 & 120 min). The amount of phosphorylation of MEK1/2 was determined using western immunoblotting with specific anti-phospho MEK1/2 antibody and densitometry readings.

Results

M. tuberculosis H37Rv induced activation of MEK1/2 at 30 min, producing peak phosphorylation at 45 min (~3.5-fold increase) and the activation signal declined to basal level at 60 min. LPS began its activation at 45 min, peaked at 60 min (~2.5-fold) and reached basal level at 120 min. Activation of Beijing and 1338 strains peaked at 120 min (~2 & 3.5-fold) before which, they had very minimal activation till 60 min. In contrast, strains *M. tuberculosis* H37Ra and 2567 induced phosphorylation as early as 15 min, signal dipped slightly at 45 min and peaked again at 60 min (~3.5 & 3-fold). While the activation signal of *M. tuberculosis* H37Ra had dropped to basal level at 120 min, the phosphorylation signal of 2567 strain still remained high at 120 min (~2.5-fold). Total MEK levels remained consistent throughout the infections, indicating that phosphorylation was specific to the external stimuli by the mycobacteria (Fig.8 A & B). The activation kinetics of MEK1/2 by all the five strains has been different.

Fig.8: MEK1/2 MAPK activation of THP-1 human monocytes in response to infection with different live *M. tuberculosis* strains

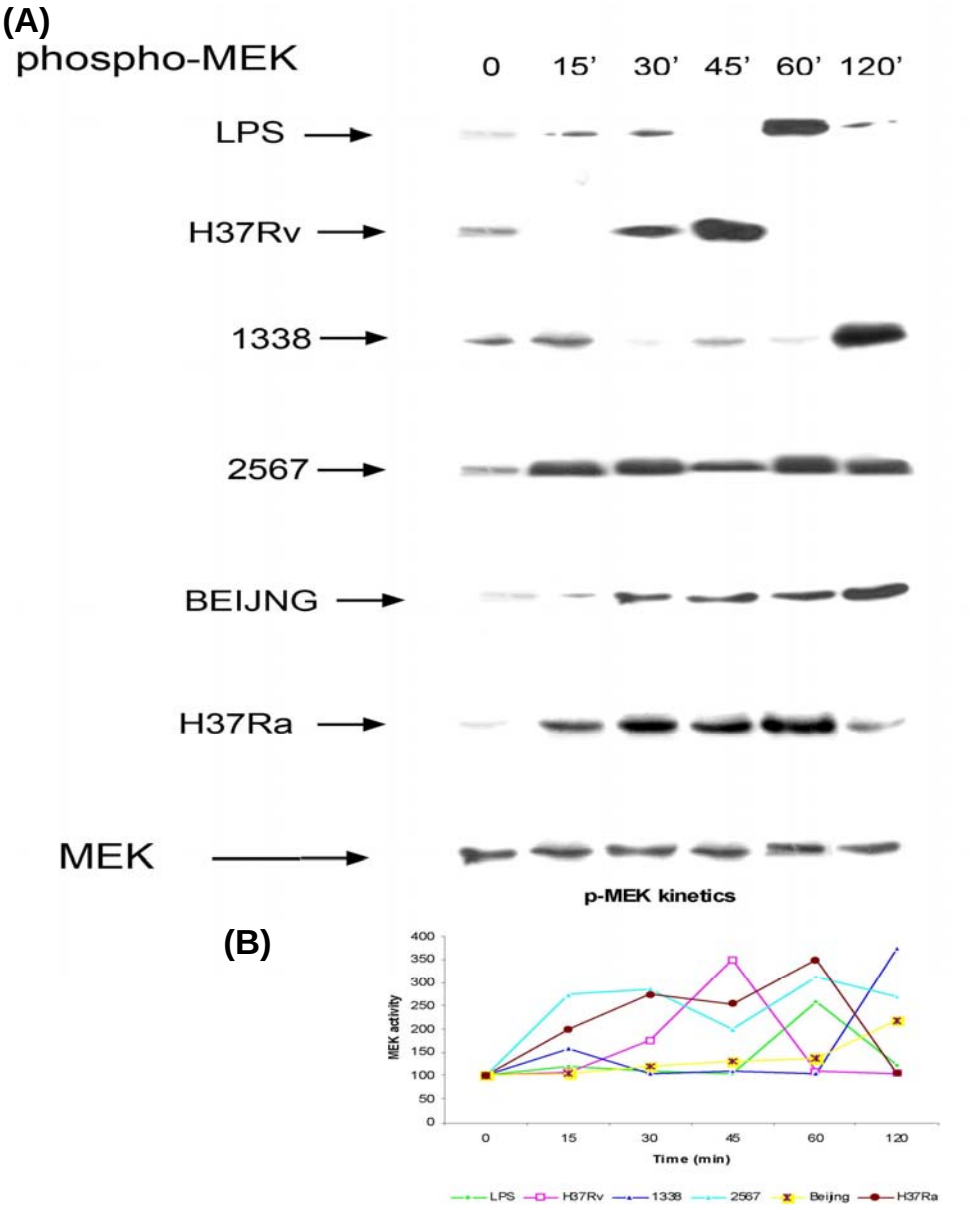


Fig.8 (A) Each of the blots was also probed with antibodies against total MEK to ensure equal loading of protein in all the lanes (represented as MEK at bottom).
Fig.8 (B) Corresponding densitometric analyses of blots probed with antiphospho-MEK1/2 antibody. Data shown are the mean of three independent experiments performed in triplicate.

Future work

The activation kinetics of p38, JNK, ERK, MKK3/6 in response to infection with the different strains of *M. tuberculosis* will be carried out.

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

***M.tuberculosis* groE promoter controls expression of bicistronic groESL operon and shows differential regulation under stress conditions**

Background

Heat shock promoters of mycobacteria are strong promoters which get rapidly upregulated during macrophage infection and thus serve as valuable candidates for expressing foreign antigens in recombinant BCG vaccine. Heat shock response is characterised by global transcriptional changes including elevated expression of a set of highly conserved genes in response to exposure to a sudden increase in ambient temperature and stress. The mycobacterial Hsp family includes proteins like dnaK, dnaJ, grpE, groES, groEL and other low molecular weight heat shock proteins. Most of these proteins have house-keeping functions and are essential for survival. dnaK, dnaJ, groES, groEL1, groEL2 and acr1 proteins are well characterised immunodominant antigens of mycobacteria and serve as excellent vaccine candidates

Unlike other bacteria, mycobacteria possess two groEL (groEL1 and groEL2) genes, out of which, the groEL1 is present downstream to that of the groES gene, while groEL2 is present at a different location and is monocistronic. In all mycobacterial species for which genome sequences are available, the organization of groES and groEL1 is identical- upstream groES and downstream groEL1. Thus, it is tempting to speculate that as in other bacteria, groES and groEL1 may form an operon in mycobacteria. But published report has shown using primer extension analysis that groEL1 gene is not co-transcribed with the upstream groES gene, and argued against the operonic organization of groES and groEL1 genes in mycobacteria.

Aims

- Characterization of the groE promoter of *M. tuberculosis*
- To determine the promoter region by deletion
- To identify the transcriptional start site (TSS) by primer extension analysis
- To confirm if groESL1 forms a bicistronic operon by northern blot and RT-PCR
- To explore differential regulation under stress conditions

Methods

The upstream promoter sequences of groES gene as well as groES-EL1 intergenic sequence were PCR amplified and cloned in pJEM13. The promoter activity was evaluated by beta-galactosidase assay. The activity of the different upstream promoter constructs were evaluated by beta-galactosidase assay under different stress conditions such as temperature shock (42^{o}C and 4^{o}C), pH stress (pH 4 and pH 9), oxidative stress (H_2O_2), sodium dodecyl sulphate (SDS) stress, osmotic and dehydration stress conditions.

Total RNA was isolated from *M. tuberculosis* and *M. smegmatis* by Trizol and bead beating method. Primer extension analysis was carried out using MoMuLV RNase H-reverse transcriptase. Heat shock experiment was done by growing *M. tuberculosis* cultures at 37°C till mid-logarithmic phase and then shifting it to 42°C for 1 hr and then shifting back to 30°C for 1hr and reshifting the culture to 42°C for 1hr. Aliquots of culture were taken at 37°C, 42°C upshift, 30°C downshift and final 42°C upshift, and RNA was isolated.

Northern blot analysis was carried out on *M. tuberculosis* total RNA (50µg) using radiolabelled probe spanning *groES* ORF along with its promoter and downstream sequence by using Rediprime labeling kit, followed by autoradiography.

RT-PCR was done on *M. tuberculosis* total RNA (1µg) by reverse transcription into cDNA using MoMuLV RNase H-reverse transcriptase enzyme at 42°C, for 1hr, using random hexamer primer, followed by PCR amplification using specific primers. For negative control, reverse transcriptase was omitted in the reaction

mixture. The RT-PCR product was subjected to electrophoresis on 1.5% agarose gel.

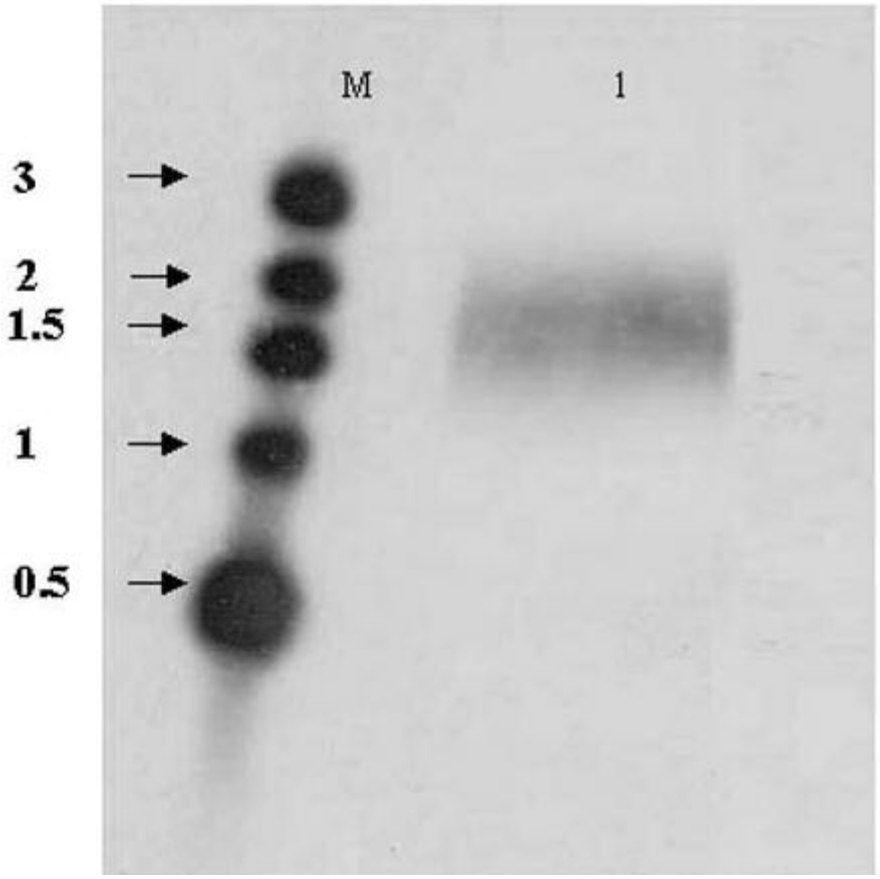
Results

Northern blot analysis was done to confirm the bi-cistronic nature of the *groE* operon. The groE promoter, groES ORF and the groES-groEL1 intergenic spacer were amplified as a single PCR product and were used as a probe. This probe identified a 2 kb transcript which is consistent with the size predicted for the bi-cistronic groESL operon (Fig.9). Further, the same RNA yielded products of sizes 412 bp and 712 bp, by RT-PCR, with primers va3-gel1R2 and ups3ap1-gel1R2, clearly indicating the presence of the groESL operon (Fig.10). Primer extension analysis identified two transcriptional start sites, namely, TSS1 (-236) and TSS2 (-171) out of which one (TSS2) was heat-inducible (Fig.11). Heat shock experiment suggested that the promoter is upregulated at 42^{o}C and gets repressed when shifted to 30^{o}C. Similar effect is not observed for groEL1 transcripts. The groE promoter identified in the study was found to be more active than the groEL2 promoter in mycobacteria. Further, it was found to be differentially regulated under stress conditions, while the groEL2 promoter was constitutive.

Future Work

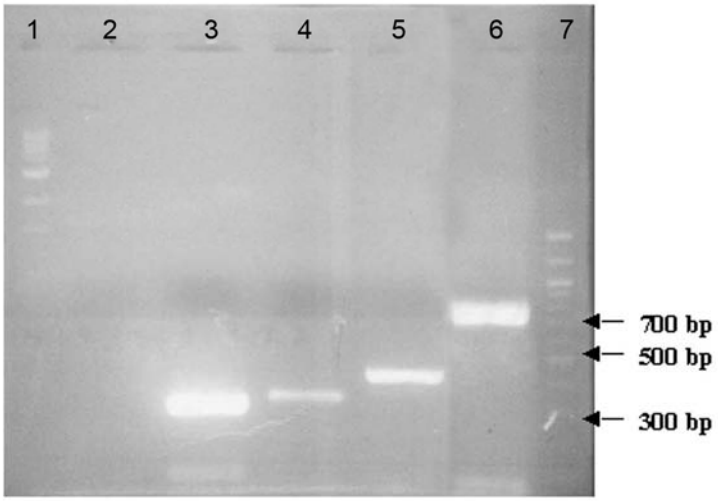
- To study the regulation of the groE promoter *in vivo*
- To confirm the CIRCE binding elements by EMSA

Fig.9: Northern blot analysis showing the 2 kb bis-cistronic mRNA transcript of groESL operon



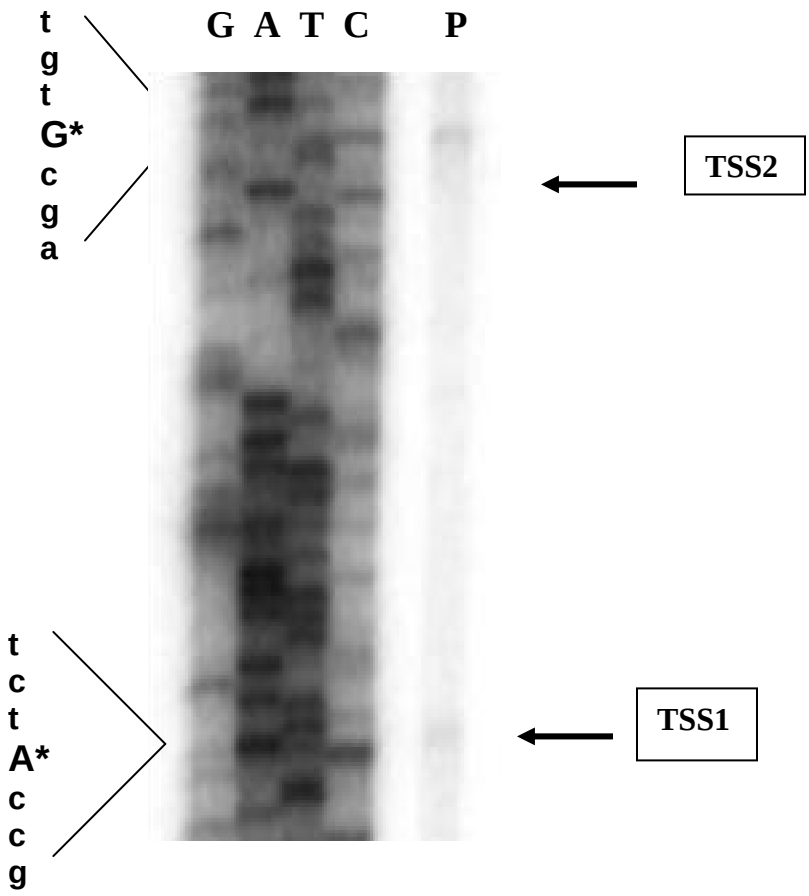
1 µg of *M. tuberculosis* RNA was fractionated in 1% agrose gel, transferred to a nylon membrane and probed with radiolabelled groES PCR product

Fig.10: RT-PCR analysis on total RNA isolated from *M. tuberculosis*



Lane 1, 1kb Marker (NEB), Lane 2, -RT; Lane3, ups3ap1-upsrkp1 (300 bp); Lane 4, va3- va5 (312bp); Lane 5, va3- gel1R2 (412 bp); Lane 6, ups3ap1-gel1R2 (712 bp) and Lane 7, 100 bp ladder

Fig.11: Identification of groESL TSS by primer extension analysis



Primer extension and DNA sequencing products (Lanes, A, C, G & T) were generated by end-labelled primer. The products were separated on 6% urea-polyacryalamide sequencing gel.

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

Global transcriptome profile of PknE deletion mutant from *M. tuberculosis* during THP-1 human macrophage infection

Background

Pathogenicity of mycobacteria is linked to survival within the macrophages in a bactericidal environment. Modulation of host cellular trafficking pathways may be influenced by signal transduction molecules expressed by pathogenic bacteria.

The advancement in genomics revealed 11 eukaryotic-like serine/threonine protein kinases [STPK] in *M. tuberculosis* genome besides two component system, which perceives the signals to external milieu. We have undertaken studies to characterize the STPK PknE. In the previous report we have showed that the gene disrupted mutant induced profound apoptosis and secretion of pro-inflammamtory cytokines was impaired.

Aim

- To study the global transcriptome response between the gene-disrupted and wild type ($H_{37}Rv$) strains infected human macrophage-like THP-1 cells.

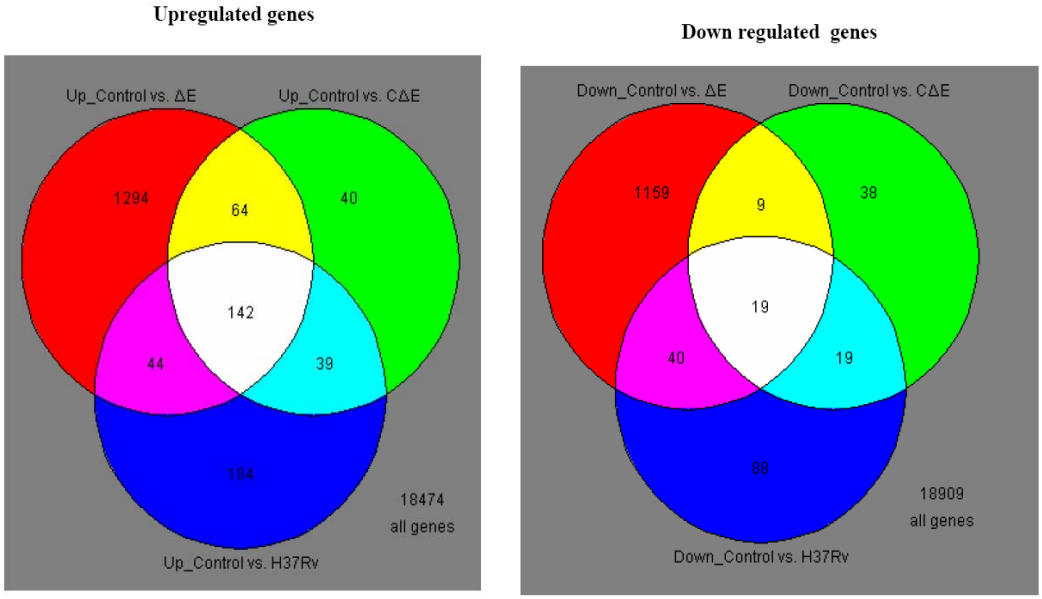
Methods

THP-1, a human monocytic cell line was differentiated into adherent macrophages by the addition of phorbol myrstate acetate (PMA). The macrophages were infected with the strains $H_{37}Rv$, ΔPknE, complemented ΔPknE. RNA was isolated from uninfected and infected cells after 5 days post infection. The quality of RNA was assessed by NanoDrop® ND-1000 spectrophotometer and Agilent 2100 bioanalyzer. The 22k human microarray was performed in an agilent platform. The data was analyzed using gene spring software.

Results

The experiment revealed enormous genes that were modulated upon the peak time point of apoptosis (Fig.12). The mutant infected macrophage showed differential expression in the gene families of our interest viz signal transduction (MAPK, JAK-STAT), apoptosis family, immune response genes (cytokines, chemokines) and metabolism. In a holistic view, the results showed that this gene was involved in altering the initial bacilli–host interaction.

Fig.12: Venn diagrams summarizing the gene expression observed in the experiment



Summary Venn diagrams:

142 Genes upregulated in all experiments

1294 Genes upregulated in experiments Control vs. ΔE

44 Genes upregulated in experiment Control vs. ΔE and Control vs. H37Rv

184 Genes upregulated in experiment Control vs. H37Rv

39 Genes upregulated in experiment Control vs. H37Rv and Control vs. CΔE

40 Genes upregulated in experiment Control vs. CΔE

64 Genes upregulated in experiments Control vs. ΔE and Control vs. CΔE

Summary Venn diagrams:

19 Genes downregulated in all experiments

1159 Genes downregulated in experiments Control vs. ΔE

40 Genes downregulated in experiment Control vs. ΔE and Control vs. H37Rv

88 Genes downregulated in experiment Control vs. H37Rv

19 Genes downregulated in experiment Control vs. H37Rv and Control vs. CΔE

38 Genes downregulated in experiment Control vs. CΔE

9 Genes downregulated in experiments Control vs. ΔE and Control vs. CΔE

Conclusion

The mutant showed modulatory effects on the host genes involved in apoptosis, immune response, metabolism and signal transduction. Hence it can be predicted that upon infection, PknE is involved in bacilli survival.

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

Characterization of FtsY, a signal particle receptor of *M. tuberculosis*

Background

The ability of *M. tuberculosis* to survive, establish and damage the host tissue depends on multiple factors like cell wall components, secreted proteins and enzymes. The signal recognition particle (SRP) pathway is a universally conserved pathway for co-translational secretion of proteins across membrane. It consists of a cytoplasmic SRP and its membrane bound cognate receptor FtsY (SR). It has already been well established that signal recognition pathway is involved in secretion in streptomyces and bacillus. There is also recent evidence to show that this pathway is required for virulence in *Streptococcus pyogenes*. The role of SRP pathway has not yet been explored in *M. tuberculosis*.

Aim

- To characterize the role of the SRP receptor FtsY of *M. tuberculosis*

Method:

Control (*M.smegmatis* mc^2 155 carrying multicopy plasmid pMAC206) and MsRvF (*M.smegmatis mc2 155* overxpressing *M. tuberculosis* FtsY under inducible acetamide promoter of pMAC206) was grown in LB medium in presence of kanamycin and induced with 0.2% acetamide for 36 hrs. The cells were pelleted, sonicated and cleared by centrifugation. Cleared lysate was ultracentrifuged at 40000rpm for 1 hr. The cytosolic and membrane fractions were run on 10% SDS PAGE and confirmed by western blot using anti C-terminal His antibody.

For colony morphology studies, 10μl of saturated culture of the strains were spotted on 7H9 plates supplemented with 0.2% acetamide and incubated at 37°C for 4-5 days.

Results

Upon resolving the membrane and cytosolic fraction of crude lysate obtained from MsRvF in 10% SDS-PAGE gel, it was found that majority of the proteins were localized in the membrane fraction (Figs.13 & 14).

Colony morphology studies showed that the control strains strains grew as smooth, shining, flat, well spread colonies with irregular edges whereas the colonies of MsRvF strains were small, more compact, raised, rough and had highly wrinkled surface (Fig.15).

Discussion

Like FtsY of other prokaryotes FtsY of *M. tuberculosis* is found to be localized in membrane region of *M.smegmatis* mc^2155 upon overexpression. In order to study the phenotypic characteristics of the FtsY gene, it was heterologously overexpressed in *M.smegmatis*. Though it did not affect the overall growth (data not shown), there was considerable difference in colony morphology between the two strains when grown on solid media. The rough and wrinkled surface of MsRvF indicates that over expression of FtsY leads to changes in the cell wall properties most importantly the glycopeptidolipids on the cell surface. These lipids play a major role in the entry of pathogen into host cells and also in biofilm formation.CF MF

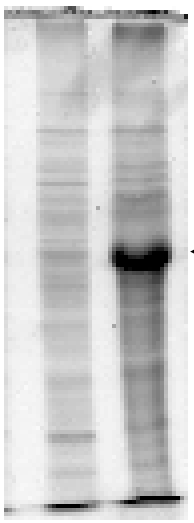


Fig.13: Localization of the acetamide induced *M. tuberculoiss* FtsY protein *in M.smegmatis* by differential

centrifugation partitioning CF, Cytosolic fraction: MF, Membrane

Fig.14: Western blot analysis of localization of the acetamide induced *M. tuberculosis* FtsY protein in *M.smegmatis* by differential centrifugation partitioning. CF cytosolic fraction MF membrane fraction

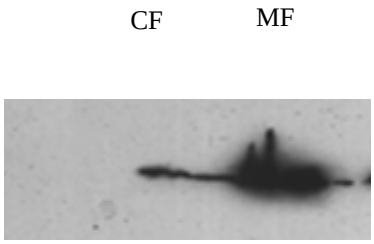
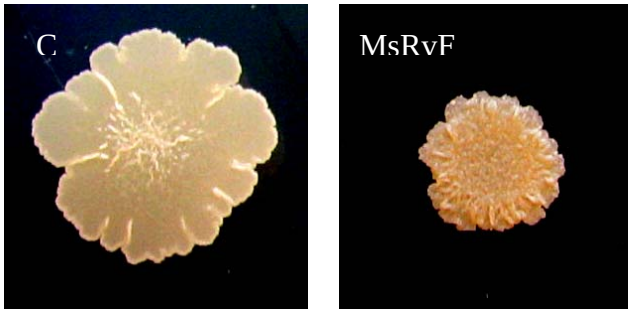


Fig.15: Colony morphology of Control (C) and MsRvF (M) 10μl of saturated culture of control and MsRvF were spotted on 0.8% 7H9 agar supplemented with 0.2% acetamide were grown at 37°C for 4-5 days



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

Ongoing studies

Role of variant genotypes of VDR gene on plasma vitamin D_3, VDR expression and intracellular cytokine positive cells in pulmonary TB

Background

Our earlier studies revealed that VDR gene variants regulate macrophage phagocytosis, lymphocyte function and various cytokine responses to *M. tuberculosis* antigens in normal healthy subjects and pulmonary TB patients. Studying the role of variant genotypes of VDR on plasma vitamin D_3 level, VDR expression and intracellular cytokine positive cells will explore the basic molecular events associated with vitamin D_3 and immunity to TB.

Aim

- To study the regulatory role of variant genotypes of VDR gene on plasma vitamin D_3, VDR expression and intracellular cytokine positive cells in pulmonary TB

Methods

The study population comprised of 70 PTB and 70 NHS. Enumeration of T-cell subsets positive for TNF-α and IFN-γ is being done in peripheral blood mononuclear cell (PBMC) cultures by flow cytometry at various time points. Portion of the PBMCs is used for VDR protein assay and DNA extraction for genotyping of VDR. Plasma vitamin D_3 is estimated using commercial ELISA kits.

Results

The effect of vitamin D_3 on IFN-γ and TNF-α cytokine expression at the intracellular level, VDR expression and vitamin D_3 levels has been studied in 15 NHS and 15 PTB patients so far. A significant suppressive effect of vitamin D_3 was observed on IFN-γ and TNF-α expression in CD4 and CD8 positive cells stimulated with live M.*tuberculosis* and CFA ($p<0.05$). A significantly increased level of 1,25 dihydroxy vitamin D_3 was observed in PTB patients compared to NHS ($p=0.007$) and a decreased VDR protein level (femtomoles/mg total protein) was observed in PTB patients as compared to NHS ($p=0.03$). More number of samples will be studied.

The study is in progress.

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

Molecular subtyping of HLA -A11, -B40 and -DR2 antigens in HIV and HIV-TB patients of south India

Background

Our earlier studies revealed the association of HLA-A11 with resistance while HLA-B40 and -DR2 with susceptibility to HIV and HIV-TB. Variability among HLA subtypes are known to influence HIV/AIDS differentially. Hence identification of specific subtype of HLA-A11, -B40 and -DR2 antigens are sought to dissect the role of HLA in influencing HIV and HIV-TB in south Indians.

Aim

- To identify the allelic subtypes of HLA-A11, -B40 and -DR2 antigens that may be associated with susceptibility or resistance to HIV and HIV-TB in a south Indian population

Methods

Molecular subtyping for HLA-A11, -B40 and -DR2 positive subjects among HIV+TB-, HIV+PTB+, HIV-PTB+ and healthy controls is carried out employing PCR based sequence specific oligonucleotide probe method and detection by chemiluminescence.

Results

The preliminary results suggest that HLA-A*1101 is the predominant subtype among HLA-A11.

Further experiments are in progress.

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

CD209 gene polymorphisms in HIV and HIV-TB patients of south India

Background

Our earlier study has shown that variant genotypes and diplotypes of mannose binding lectin gene are associated with susceptibility to development of TB in HIV-1 infected patients. Polymorphisms in CD209 gene are known to influence immune responses and might be associated with susceptibility to HIV-1 infection and development of TB in HIV-1 infected patients.

Aim

- To find out whether polymorphisms in CD209 genes are associated with susceptibility or resistance to HIV and HIV-TB

Methods

The study subjects include 131 HIV-positive TB-negative patients (HIV+TB-), 82 HIV-positive patients with pulmonary TB (HIV+PTB+), 107 HIV-negative PTB positive patients (HIV-PTB+) and 146 healthy controls. CD209 exon 4 repeat polymorphism was studied by using polymerase chain reaction method.

Results

The allele 7.5 was found to be the most frequent repeat allele of CD209 exon 4 repeat polymorphism observed in the study subjects with a frequency of more than 99%.

The study is in progress.

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

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

Background

Immunity to TB is mediated through T-cells and information on the molecules recognized by the cellular subsets mediating protection is still scarce. The culture filtrate (CF) from *M. tuberculosis* was fractionated by preparative two-dimensional electrophoresis to identify the antigens that strongly stimulate T-cells.

In previous years' Annual Report (2005-2006 and 2006-2007), a systematic approach was adopted to test the antigens purified by two-dimensional preparative separations,

in human subjects and the results of *in vitro* assays have been described. Significantly higher ($p<0.05$) IFN-γ secretion was induced by 105 fractions in healthy household contacts (HHC) compared to TB patients.

All these fractions were subjected to proteomic analysis using tandem mass spectrometry (ESI LC MS/MS) and characterization of the fractions are presented in this report

Aims

- To identify a set of immunologically relevant T-cell antigens
- To evaluate the response to these antigens in patients with TB and controls

Methods

The study subjects are as follows:

- Apparently HHC of sputum positive pulmonary TB living in the same household (TB was ruled out in this group during the time blood collection and hence considered "Protected")
- Newly diagnosed adult pulmonary TB cases and they form the "susceptible" group

The methods followed are as follows:

Five microgram of protein from each fraction was subjected to overnight digestion with 1µg of trypsin. All the samples were analyzed using Thermo Finnigan LTQ mass spectrometer at Dr. John T. Belisle's laboratory at Colorado State University, USA.

Results

The data from the protein digestion analyzed by LC MS/MS were searched against the *M. tuberculosis* proteome database using SEQUEST software. All the peptides were assigned to the gene products of *M. tuberculosis*, based on the experimentally derived MS/MS fragmentation pattern. Protein identification by SEQUEST was further validated by Scaffold software. In the 105 fractions which induced significant IFN-γ response in contacts, 57 different proteins were identified. Among these proteins, 35 proteins observed as immunodominant antigens were reported in earlier studies and 22 proteins were novel T-cell antigens identified in our study (table 20).

Generally, in literature low molecular weight proteins (<35kDa) have been described as immunologically active. Our study has identified 12 potential T-cell protein antigens

which have a molecular weight >40kDa (FabG4, GgtB, GlnA1 MmsA, Pks13, ProA, Rv0462, Rv2251, Rv2721c, Rv3169, SahH, and Tal).

Some of the novel antigens identified in this study, are being over-expressed as recombinant proteins in *E. coli* and immunologically characterized, so that their potential in subunit vaccine design and specific diagnosis of TB can be evaluated.

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

Table 20: Novel T-cell antigens identified in IFN-γ inducing 2-D liquid phase electrophoresis fractions of the culture filtrate protein

Protein name	Rv no	Biological function
Can	Rv1475c	TCA cycle aconitase enzyme
AcpM	Rv2244	Involved in fatty acid bioynthesis
Adk	Rv0733	ATP AMP transphosphorlase(adenylate kinase)
Ald	Rv2780	Secreted L-alanine dehydrogenase
FabG4	Rv0242c	Probable 3-oxoacyl-(acyl-carrier protein) reductase
Fba	Rv0363c	Involved in glycolysis (fructose bisphophate aldolase)
Frr	Rv2882c	Ribosomal recyclic factor
GgtB	Rv2394	Probable gamma glutamyl transpeptidase precursor
MmsA	Rv0753c	Probable methylmalonate-semialdehyde dehydrogenase
Pgi	Rv0946c	Probable glucose 6 phosphate isomerse
Pks13	Rv3800c	Polyketide synthase
ProA	Rv2427c	Probable gamma glutamyl phophate reductase protein
Rv1324c	Rv1324c	Possible thioredoxin
Rv1558	Rv1558	Function unknown
Rv1910c	Rv1910c	Function unknown
Rv2204c	Rv2204c	Function unknown
Rv2721c	Rv2721c	Function unknown
Rv3169	Rv3169	Function unknown
Rv3716c	Rv3716c	Function unknown
SahH	Rv3248c	S-adenosyl L-homocysteine hydrolase
Tal	Rv1448c	Probable trans aldolase
TB49.2 or CIP50	Rv0462	Probable protein involved in the phagosomal maturation arrest

Cytotoxic cell response in *M. tuberculosis* infection

Background

Early secreted antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are antigens encoded by ESX-1 locus of *M. tuberculosis*, but absent in *M. bovis* BCG and are

useful in diagnosis of tuberculous infection, as well as when incorporated in vaccines. ESAT-6 and CFP-10 are potent T-cell antigens. Studying the role played by specific T-cell subsets in response to overlapping peptides may reveal epitopes in these proteins which might be useful in diagnosis and vaccine design.

Aim

- To investigate the IFN-γ and IL-4 responses of CD4 as well as CD8 T-cell responses to overlapping peptides (20-mers overlapping by 10 amino acids) of both ESAT-6 and CFP-10

Methods

The two study groups of this study were healthy household contacts (HHC) (n=20) and pulmonary TB patients (PTB) (n=20).

The IFN-γ as well as IL-4 responses to all the overlapping ESAT-6 peptides and CFP-10 peptides were studied. Diluted whole blood (1:2) of subjects was cultured with the respective ESAT-6, CFP-10 peptides and whole antigens. The intracellular cytokine response was investigated by flow cytometry.

Results

In the previous Annual Report (2006-2007), lymphocyte proliferation to ESAT-6, CFP-10 peptides was shown. We observed peptides Esp1, Esp6, Esp7 and Esp8 among ESAT-6 and the peptides Cfp6, Cfp7, Cfp8 and Cfp9 among CFP-10, to induce more CD4 and CD8 cell proliferation in HHC than PTB. In this report, cytokine production to ESAT-6 and CFP-10 overlapping peptides has been investigated.

The percentage of IFN-γ and IL-4 positive CD4 and CD8 cell induced by the overlapping peptides of both the proteins was measured. When IFN-γ responses in CD4 cells to ESAT-6 peptides was considered, it was found that only the peptides $Esp1_{1-20}$ (***$p<0.001$) and $Esp6_{51-70}$ (**$p<0.01$) induced a significantly higher percentage of CD4 positive cells in HHC when compared to PTB. For the whole ESAT-6 protein the response was significantly elevated in HHC compared to PTB (***$p<0.001$) (Fig.16).

The IFN-γ responses were also studied in CD8 cells to ESAT-6 peptides. It was observed that as in the case of CD4 cells, the peptides Esp1 (***$p<0.001$) and Esp6

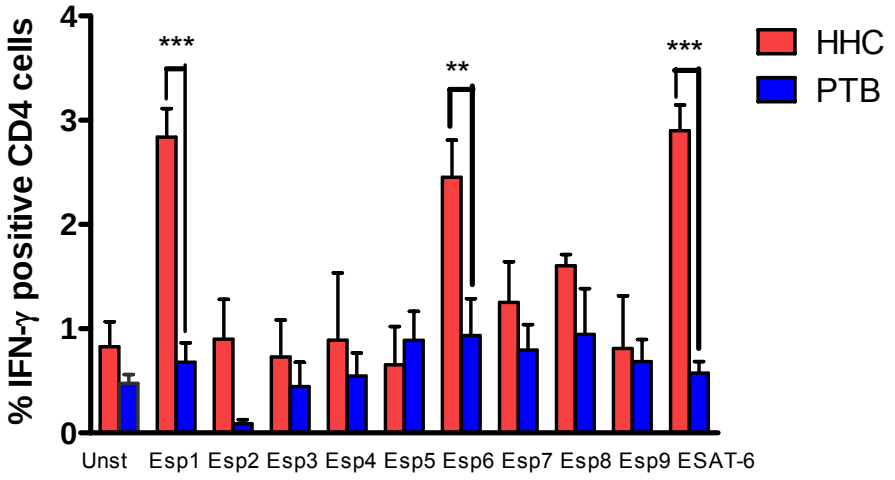
(**p<0.01) induced significantly higher percentage of IFN-γ positive CD8 T cells in HHC compared to PTB. No significantly enhanced response was seen for whole CFP-10 protein between HHC and PTB (Fig.17).

Since peptides inducing Th1 cytokines to a greater extent and Th2 cytokines to a lesser extent are considered to be protective, the ratio of IFN-γ to IL-4 was studied for all the ESAT-6 peptides. Of all the peptides, the peptide Esp6 induced an enhanced response (*p<0.05) in HHC when compared to PTB. For rest of the peptides and whole ESAT-6 protein, no significant difference in response was observed (Fig.18).

The IFN-γ responses to CFP-10 peptides were studied in CD4 as well as CD8 cells. None of the peptides exhibited a significant response for both the cell types (data not shown). IFN-γ /IL-4 ratio was studied in CD4 and CD8 cells for all the CFP-10 peptides. Although an increased response was observed for the peptides Cfp6 $_{51-70}$, Cfp7 $_{61-80}$ and Cfp8 $_{71-90}$ in HHC and not in PTB, this was not significant (Fig.19).

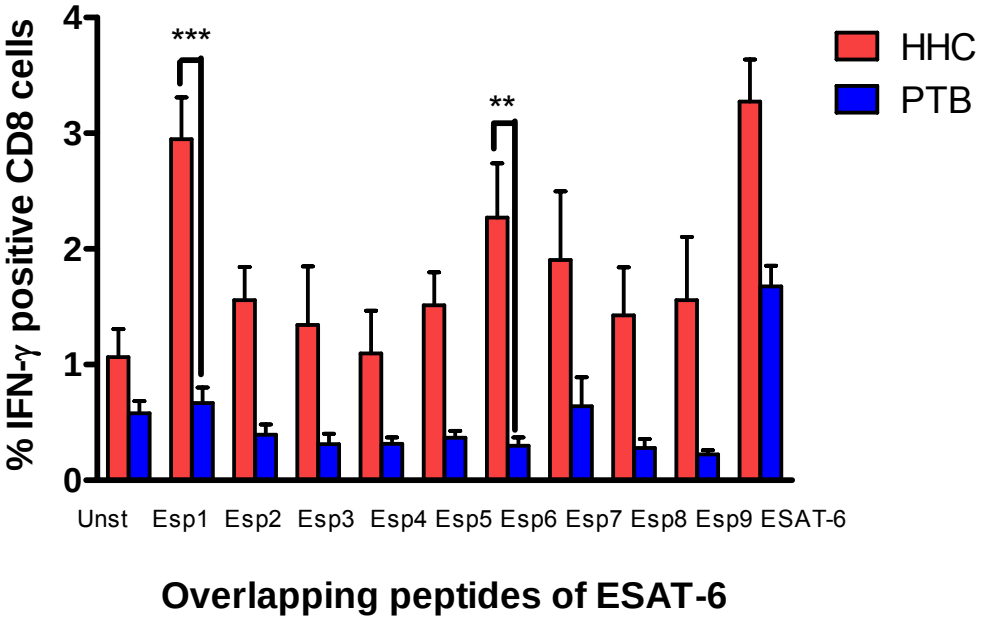
The ESAT-6 peptide, Esp6$_{51-70}$ evinced an enhanced IFN-γ /IL-4 ratio in HHC manifesting that it contains a protective epitope which may have implications in vaccine design. None of the CFP-10 peptides displayed a protective response.

Fig.16: Intracellular IFN-γ response to ESAT-6 overlapping peptides in CD4 cells



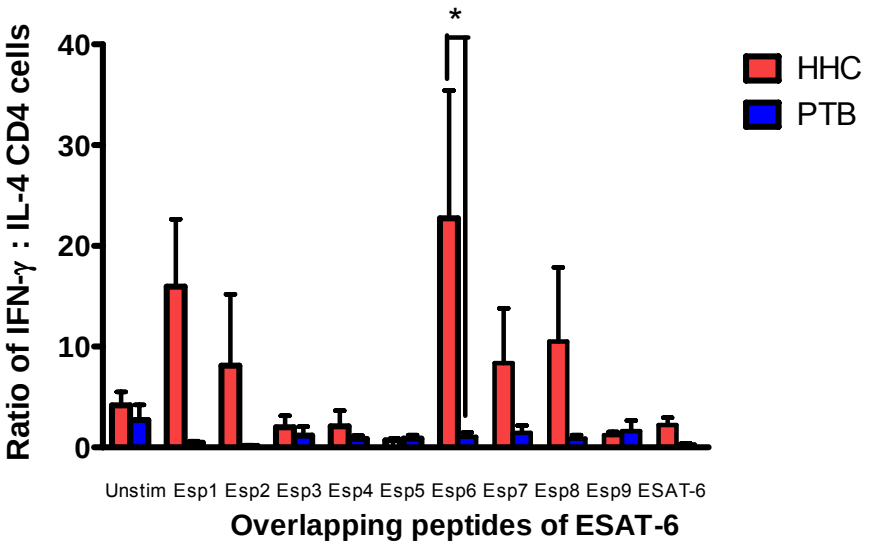
Statistical analysis between groups was done by one way ANOVA.
Significance between groups was determined by Bonferroni's post test. (*p<0.05, **p<0.01, **p<0.001)

Fig.17: Intracellular IFN-γ response to ESAT-6 overlapping peptides in CD8 cells



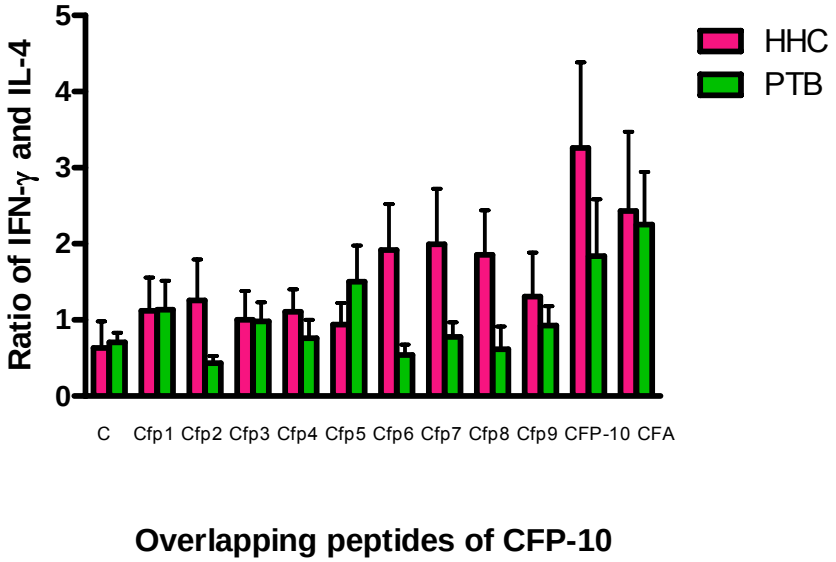
Statistical analysis between groups was done by one way ANOVA.
Significance between groups was determined by Bonferroni's post test. (*p<0.05, **p<0.01, **p<0.001)

Fig.18: IFN-γ /IL-4 ratio to overlapping peptides of ESAT-6 in CD4 cells



Statistical analysis between groups was done by one way ANOVA.
Significance between groups was determined by Bonferroni's post test. (*p<0.05, **p<0.01, **p<0.001)

Fig.19: IFN-γ /IL-4 ratio to overlapping peptides of CFP-10 in CD4 cells



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

Innate immunity in HIV infection

Background

HIV-infected individuals are subjected to various immune disorders, particularly when co-infected with TB disease. Natural Killer (NK) cells are characterized by potent cytotoxic activity against tumors, virus-infected cells and intracellular parasites without prior sensitization and MHC restriction. Defective NK cell functions are among the various immunological abnormalities in HIV infection and are known to be partially restored *in vitro* by IL -2 and IL-12. IL-15 shares receptor and several biological properties with IL-2. A study was undertaken to look at the effect of stimulants such as *M. tuberculosis* and cytokines IL-2, IL-12 and IL-15 on the expression of various cytokines. NK cytotoxicity upon *in vitro* stimulation was also studied.

Aim

- To demonstrate NK cell mediated innate immune response in HIV-TB

Methods

Study groups included normal healthy subjects (NHS n=15), patients with pulmonary TB (TB n=15), HIV positive subjects without TB (HIV n=15) and HIV positive patients with TB (HIV-TB n=15).

In vitro intracellular cytokine secretion (IL-2, IL-4, IFN-γ and TNF-α) response of NK cells to *M. tuberculosis* H37Rv and stimulatory cytokines (IL-15, IL-15+IL-12, IL-15+IL-2) was studied using flow cytometry. The cytotoxic activity of NK cells against tumor cell lines (K562) after *in vitro* stimulation was assessed by a flow cytometry based assay.

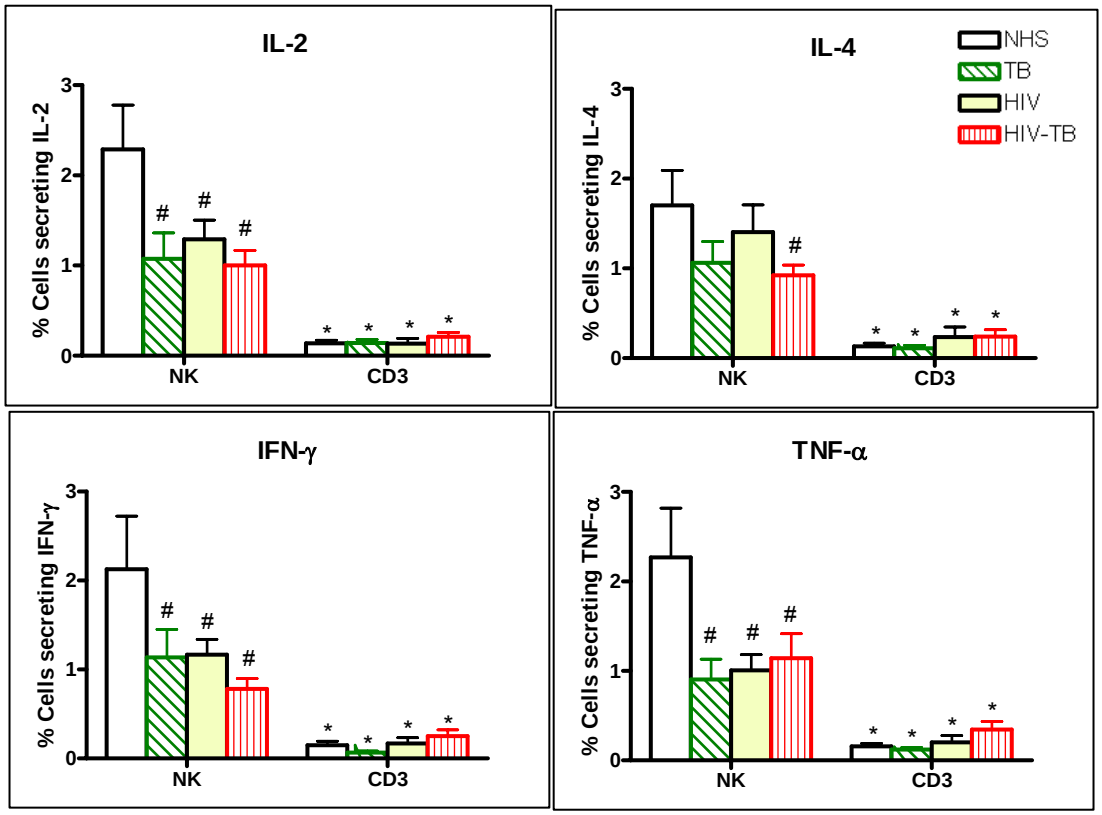
Results

Cytokine response of NK cells to *M. tuberculosis* H37Rv and stimulatory cytokines in two of the study groups, NHS and TB, were reported in Annual Report 2006-07. Results for all four groups are presented here. Basal cytokine expression pattern of lymphocytes among various groups is presented in (Fig.20). The expression of Th1 cytokines IL-2, IFN-γ and TNF-α was decreased significantly in TB, HIV and HIV-TB groups (p<0.05), when compared with NHS. No such difference in cytokine pattern was observed in CD3+

cells. The secretion of Th2 cytokine (IL-4) by NK cells was decreased only in HIV-TB when compared to NHS. When we compared cytokine expression between NK and CD3 cells, the expression of Th1 and Th2 cytokines were elevated in NK cells for all groups (p<0.05). With *M. tuberculosis* stimulation, we observed increased Th1 cytokine in NHS compared to basal response. No significant changes were observed with other groups (data not shown). Upon stimulation with IL-15+IL-12, we found increased IL-2 and IFN-γ secreting NK cells in HIV and HIV-TB group (p<0.05) when compared to NHS (Fig.21). Maximal NK cytotoxicity (p<0.05) was observed in the presence of IL-15+IL-12 combination in NHS, TB, HIV and HIV-TB (Fig.22). At 50:1 effector/target concentration, the mean increase in NK cytotoxicity upon stimulation with IL-15+IL-12 was 2-fold for HIV positive subjects and 1.8 fold for HIV-TB co-infected patients (data not shown).

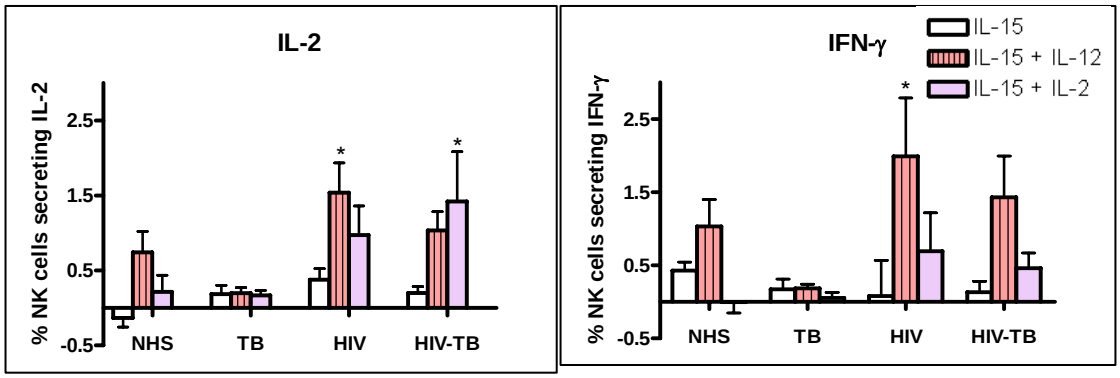
Augmentation of Th1 cytokine secreting NK cells and NK cytotoxicity after stimulation with IL-15+IL-12 in HIV positive subjects and HIV-TB co-infected patients suggests its potential in improving NK cell function.

Fig.20: Basal cytokine expression pattern of lymphocytes among various groups



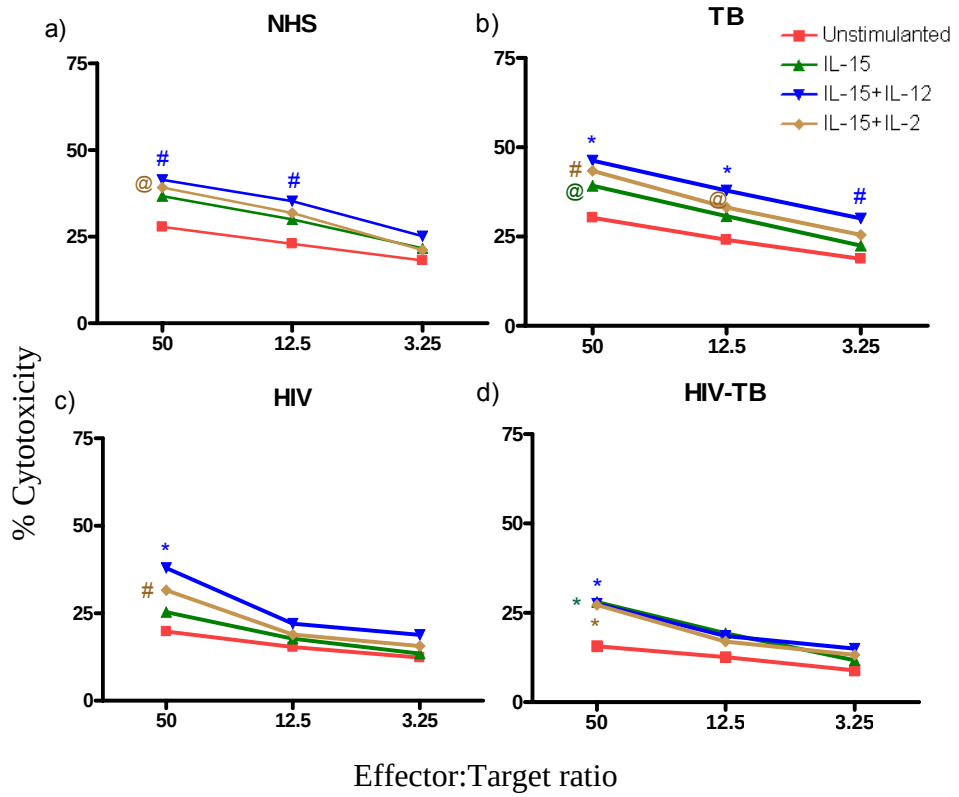
Data are represented as mean + SEM of 15 subjects. # represents the significance ($p<0.05$) when compared with NHS within one type of lymphocytes. * refers the significance ($p<0.05$) where the comparisons are between NK cells and CD3+ cells

Fig.21: NK cell response upon *in vitro* stimulation with cytokines



Values are represented as mean of 15 subjects. All the comparisons are with IL-15 stimulant. * refers to $p < 0.05$

Fig.22: NK cytotoxic response to individual groups



Values are represented as mean of 15 subjects. All the comparisions are with unstimulated. @ represents $p < 0.05$, # indicates $p < 0.01$, * refers to $p < 0.001$

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

Interferon gamma assay for latent TB in HIV infection

Background

HIV infection is a risk factor for rapid progression of a recently acquired TB infection and for reactivation of latent TB infection. Because of the associated higher risk of mortality, tests that detect *M. tuberculosis* infection and disease at early stages are needed to initiate chemoprophylaxis/therapy.

Aim

- To assess sensitivity of interferon gamma releasing assay (IGRA) for active TB diagnosis in patients with or without HIV infection

Methods

The study subjects are as follows:

- TB (N = 139)
- HIV-TB (N = 105)

TST was performed and read as per standard procedures, using 2 TU of PPD RT23 (Statens Serum Institut, Denmark) and reading was taken 48-72 hrs post testing. Whole blood IGRA was done by using Quantiferon TB Gold kit (QFT-G) (Cellestis, Victoria, Australia) as per the manufacturer's instructions.

Results

Sensitivity of IGRA in active TB patients

In last year's Annual Report (2006-2007), it was reported that the levels of IFN-γ have been measured in 139 patients. 0.35 IU/ml was set as cut-off value as described by the manufacturer. Among the 139 PTB patients, 124 were positive and 11 were negative for QFT-G. The remaining four patients showed indeterminate or invalid results. All the indeterminate results were due to poor response to mitogen. The four invalid subjects were excluded for further sensitivity analysis.

IGRA showed sensitivity of 92% in both smear positive as well as negative cases. The percentage of positivity did not vary between smear positive and negative cases. The sensitivity was consistently high even in cavitating smear positive subjects. It showed that QFT-G have the ability to identify even severe TB cases.

Among the 139 study subjects, both TST and QFT-G results were available for 89 subjects. Among them, 53 and 65 subjects showed an induration >15mm and >10mm respectively. In this study, we used 10mm cut-off value to calculate the sensitivity of TST; hence it yielded a sensitivity of 73%. In head to head comparison with QFT-G, TST showed poor agreement with the 'k' value of 0.077 (95 CI; 0.107–0.262) (table 21). However, the induration of TST and level of IFN-γ was significantly correlated in QFT-G and TST positive subjects (Fig.23). This study strongly showed that IGRA is highly sensitive and superior to TST in detecting active PTB cases. Hence, it may be used especially in smear negative cases, where the new diagnosis methods are actually needed.

Sensitivity of QFT-G and TST in HIV-TB patients

A total of 105 HIV-TB subjects were recruited for this study. The demographic profile of the study subjects is given in table 22. Of the105 subjects tested, 68 (65%) were positive and 19 (18%) were negative for QFT-G (table 23). The remaining 18 (17%) subjects showed indeterminate results. All the indeterminate or invalid results were due to poor response to PHA. With exclusion of indeterminate results, QFT-G showed the sensitivity of 78%. Among the 53 culture positive samples, with the 11 indeterminate and 37 positive results, QFT-G showed 88% sensitivity. The sensitivity of QFT-G was consistently high in both PTB (73%) and EPTB (81%) subjects.

The median total lymphocyte, CD3, CD4 and CD8 counts were significantly lower in indeterminate cases when compared to QFT-G positives (p=0.001, p=0.003, p=0.042 and p=0.022 respectively) (Fig.24). None of them significantly differed between QFT-G positive and negative cases.

Of 102 TST study results available, 34 showed ≥5mm induration for TST; hence it yielded a sensitivity of 34%. The median of total lymphocytes, CD3 cells and CD4 cell counts were significantly lower in TST negative subjects when compared to TST positive subjects.

The results clearly evidenced that the sensitivity of QFT-G was retained in HIV-TB cases and the sensitivity was also not depressed by low CD4 count. While comparing the QFT-G and TST, the latter showed low sensitivity.

The recruitment of study subjects for healthy control, healthy contact and HIV-positive groups is going on. This would be helpful to analyze the specificity as well as sensitivity of QFT-G for latent TB diagnosis in HIV negative and positive subjects.

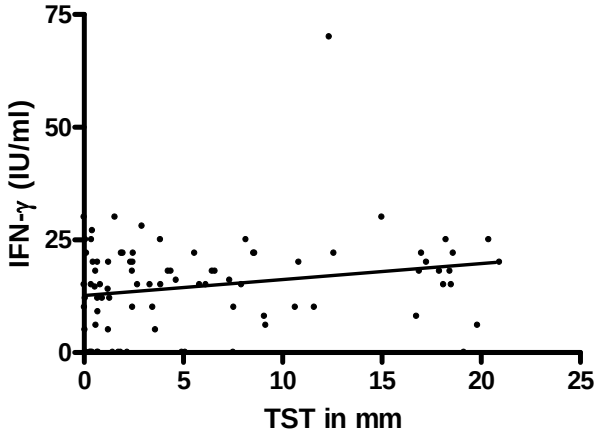
Table 21: Agreement between TST and QFT-G

		QFT-G		
		Positive	**Negative**	**Total**
TST	**Positive**	58	4	62
	Negative	21	3	24
	Total	79	7	86

The agreement between two tests was calculated by using kappa statistics.

Kappa value 0.077

Fig.23: Correlation between TST induration and magnitude of IFN-γ levels



The correlation between the magnitude of IFN-γ and TST induration was assessed by Pearson correlation analysis. R = 0.27, P = 0.01

Table 22: Demographic and baseline parameters of the 105 HIV-TB subjects

Sex, number (%)	Male	78 (74%)
	Female	27 (26%)
Age, Median in years (Range; IQR)		36 (18-63; 25, 36)
BMI, kg/m^2 (Range; IQR)		19 (13-31; 17,21)
HIV strain, Number (%)	HIV-I	89 (89%)
	HIV-I&II	11 (11%)
TB types, Number (%)	PTB	63 (60%)
	EPTB	39 (37%)
Smear result, Number (%)	Positives	47 (45%)
	Negatives	58 (55%)
Culture result, Number (%)	Positives	53 (50%)
	Negatives	52 (50%)
CD4, Median (Range; IQR) (For available 81 subjects)		116 (11-2062; 48, 209)
CD4 count, Number (%)	<100	38 (47%)
	100-199	21 (26%)

	>200	22 (27%)

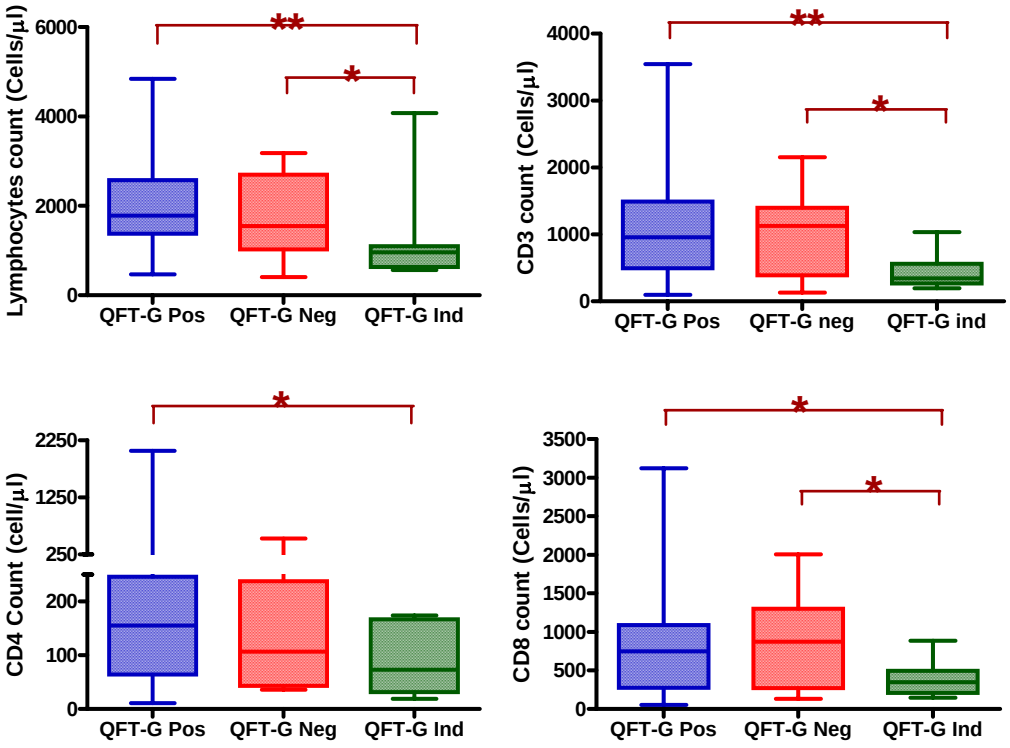
BMI - Body mass index, PTB- Pulmonary TB, EPTB - Extra pulmonary TB, IQR – Inter quartile range

Table 23: Sensitivity of QFT-G in HIIV-TB subjects

Groups	QFT-G			
	Pos (%)	**Neg (%)**	**Ind (%)**	**Sen (%)**
Overall (N=105)	68 (65)	19 (18)	18 (17)	78
Smear Positive (N=47)	32 (68)	4 (9)	11 (23)	89
Smear Negative (N=58)	37 (64)	14 (24)	7 (12)	73
Culture Positive (N=53)	37 (70)	5 (9)	11 (21)	88
Culture negative (N=52)	32 (62)	13 (25)	7 (13)	71
PTB (N=66)	39 (59)	13 (20)	14 (21)	75
EPTB (N=39)	30 (77)	5 (13)	4 (10)	86

QFT-G - Quantiferon TB Gold
Pos - Positive (Secretion of IFN-γ in response to TB antigens was >0.35 IU/ml)
Neg - Negative (Secretion of IFN-γ in response to TB antigens was <0.35 IU/ml and the mitogen response was >0.5 IU/ml)
Ind - Indeterminate (Secretion of IFN-γ in response to TB antigens was <0.35 IU/ml and the mitogen response was <0.5 IU/ml)
Sen - Sensitivity [Number of Positives/(Total subjects - Number of indeterminates)]

Fig.24: Level of total lymphocyte & T-cell count in QFT-G positive, negative & indeterminate subjects



The difference between groups was assessed using Mann-Whitney U test. * P<0.05 ** P<0.01 QFT-G - Quantiferon TB Gold, Pos – Positive, Neg – Negative, Ind - Indeterminate

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

Differential upregulation of chemokine receptors on CD56^{+} NK-cells and their transmigration to the site of infection in tuberculous pleurirtis

Background

Chemokines and their receptors orchestrate the leukocyte recruitment and confer immunity during *M. tuberculosis* infection. The immunoregulatory and cytotoxic activity of NK-cells are essential at the site of infection during tuberculous pleurisy.

Aim

To assess the frequency, subtypes, expression of phenotype markers and chemokine receptors on NK-cells in tuberculous (TB) and non tuberculous (NTB) pleural fluid (PF).

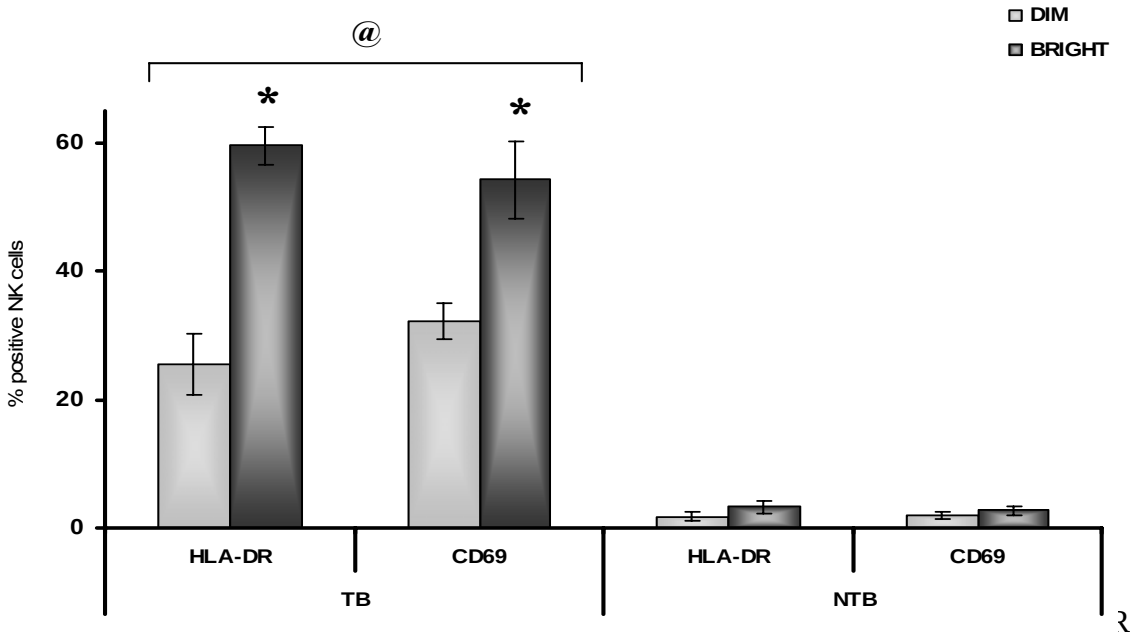
Methods

Pleural fluid mononuclear cells from TB and NTB groups were stained with CD3-FITC and CD16/56-PE antibodies to identify the NK-cell population in the lymphocyte gate by flow cytometry analysis. The NK-cell subsets were characterized for the surface expression of CD69, HLA-DR, TLR (TLR-2, TLR-4 and TLR-9) α- and β- chemokine receptors (CXCR2, CXCR3, CCR1, CCR2, CCR5 and CCR7) using CCR5 and TLR9-FITC, CCR1, CCR2, CCR7, TLR-2 and TLR4-PE, CXCR2-APC labeled mouse anti-human antibodies.

Results

A significant decrease in CD56dim with no change in CD56bright NK-cells was observed in TB group (Fig.25). Significantly increased expression of chemokine receptors CCR1, CCR2 and CCR7 on CD56bright and CCR5 on CD56dim NK-cells was also observed in TB group (Fig.26).

Fig.25: Phenotype characterization of pleural fluid NK-cell subsets in TB (N=38) & NTB (N=24) patients

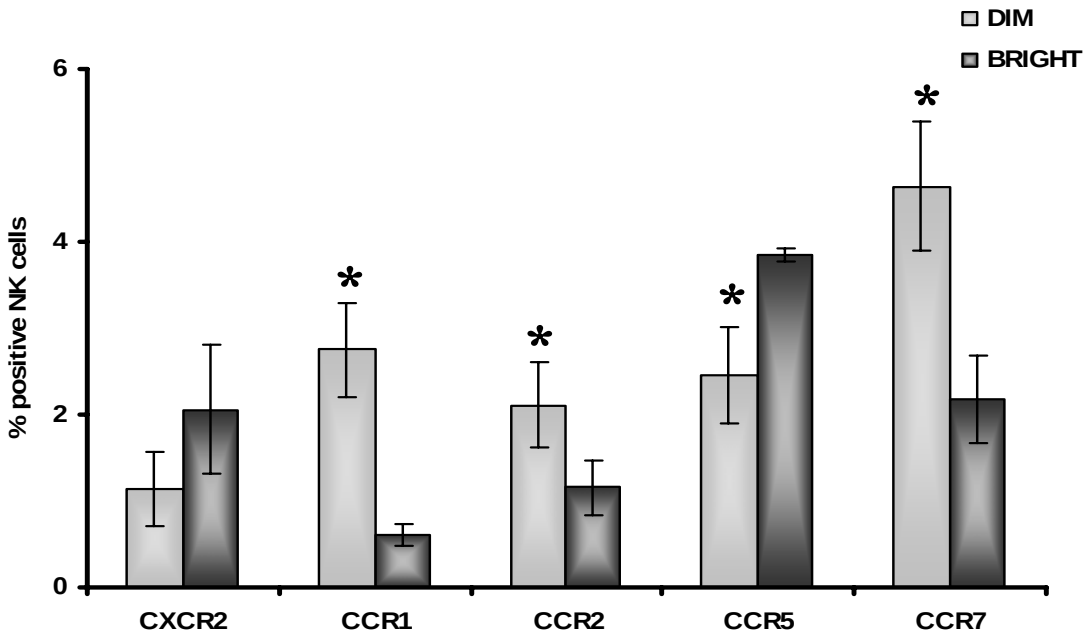


(R

and CD69 on the subsets of NK-cells in both the study groups. Results are expressed as

mean values ± standard error of mean (SEM). * @ P<0.05 was considered to be significant using independent Student's t-test and Mann–Whitney U test for comparison between groups. An isotype control was usually used to set the fluorescent compensation and to minimize the overlap of fluorochrome signals or background staining.

Fig.26: Differential expression of chemokine receptors on the subsets of NK-cells in TB



Co-expression analysis for chemokine receptors (CXCR2, CCR1, CCR2, CCR5 and CCR7) on TB and NTB PF NK-cells was performed. Results are expressed as mean values ± standard error of mean (SEM). * P<0.05 was considered to be significant using independent Student's t-test. An isotype control was usually used to set the fluorescent compensation and to minimize the overlap of fluorochrome signals or background staining.

Conclusion

The study suggests that CD56bright NK-cells may recognize *M.tuberculosis* directly using TLRs, HLA-DR and CD69. In addition, CC chemokines induce activation signals mediating differential NK-cell migration to the site. Thus NK-cells act as first direct sensors and effectors in mycobacterial infection.

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Role of dendritic cells in mycobacterial immunity - Retarded migration of *M. tuberculosis* infected dendritic cells

Background

Dendritic cells (DC) act as sentinels against pathogens in the host immune system. Their role in the initiation and regulation of the T lymphocyte response towards *M. tuberculosis* is fundamental during infection. Firstly, they are able to reach the site of infection and secondly, after recognizing the pathogen, they are activated and migrate towards lymph nodes to activate naive T lymphocytes. The movement of DC is therefore crucial in the immunological functions.

Aim

- To investigate *in vitro*, the effect of virulent *M. tuberculosis* (H37Rv) and prevalent clinical isolates of *M. tuberculosis* (S7 and S10) on the migration and chemotactic activity of DCs derived from human monocytes

Methods

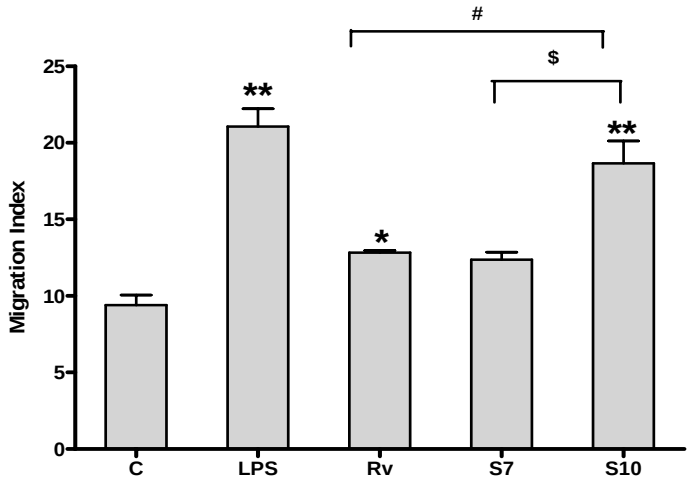
PBMCs were isolated from the blood of healthy individuals. Monocytes were purified by using anti-CD14 conjugated magnetic beads. Monocyte derived DCs (MoDCs) were generated by culturing $CD14^+$ cells with granulocyte macrophage colony stimulating factor (GM-CSF) and IL-4 for 5 days. On day 5, the MoDCs were either stimulated with LPS or infected with different *M.tuberculosis* strains. After 24 hours, DCs were harvested and analyzed using FACS for chemokine receptors (CCR5 & CCR7). ELISA for chemokines (IL-8, IP-10, MCP-1, MIP-1α) was performed in the culture supernatants. The infected MoDCs were subjected to chemotaxis assay for studying the migration activity using recombinant CCL21 (secondary lymphoid chemokine – SLC) and the chemotactic activity of infected MoDCs was performed using T lymphocytes.

Results

The levels of studied chemokines were low in *M. tuberculosis* infected MoDCs. The chemotaxis assay demonstrated suppressed migration of MoDCs after infection (Fig.27). Also the chemotaxis of T-cells against infected MoDCs, supernatants was lesser with Rv and S7 infection. The expression of CCR5 was

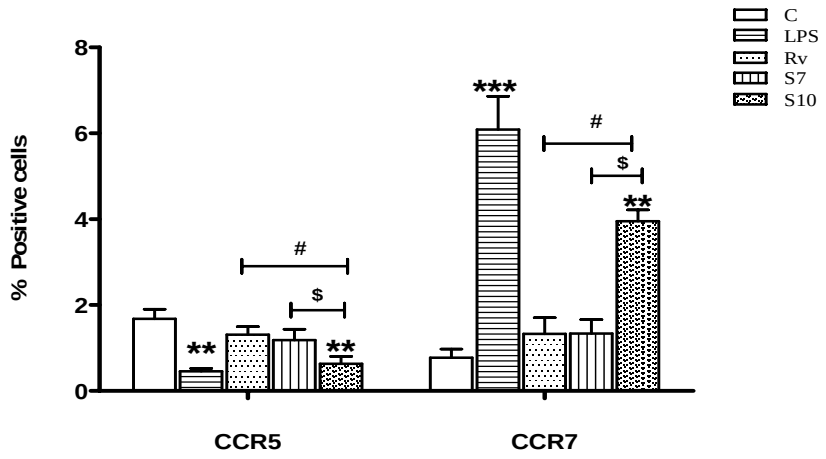
not down regulated in *M. tuberculosis* infected MoDCs as compared to LPS stimulated MoDCs, while CCR7 was not significantly upregulated with Rv and S7 infected MoDCs (Fig.28).

Fig.27: Migration of infected MoDCs to recombinant SLC



Chemotaxis of *M. tuberculosis* infected MoDCs to recombinant CCL21. The results of three independent experiments are depicted as mean ± SEM. The statistical significance is shown as * compared to uninfected control, # compared to Rv and $ compared to S7

Fig.28: Expression of chemokine receptor on infected MoDCs



Surface expression of CCR5 and CCR7 on MoDCs is shown (a) after infection with different strains of *M. tuberculosis*. Data represented as mean ± SEM obtained from ten different experiments. The statistical significance is shown as * compared to uninfected control, # compared to Rv and $ compared to S7.

Conclusion

The study findings suggest that the modulated migration of DC and its cell trafficking ability may be a potent mechanism used by *M. tuberculosis* to paralyze the early immune response of the host.

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Development of mice model of latent TB

(Funded by the Department of Biotechnology)

Background

This is a collaborative project between TRC and University of Delhi south Campus. The aim of this project is to study the immunomodulation of latent TB by recombinant strains of BCG over-expressing various *M. tuberculosis* antigens is being investigated. At TRC, the pathological changes pertaining to the disease and immunomodulation are being assessed using conventional and immuno histochemical procedures. During the period under review, the basic model for establishing latent TB using aerosol infection was standardized and the study is in progress using recombinant BCG strains.

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

(Funded by the Department of Biotechnology)

This is a collaborative project between TRC, Madurai Kamaraj University and Anna University. 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 will be used to display a highly conserved epitope from the GP41 of Human immunodeficiency virus 1. The HIV-1 epitope (aa 731-752 of GP41) harbors a neutralization domain. Expression of this epitope in multiple copies on a self-assembly system is likely to lead to an efficient vaccine against HIV. These chimeric systems that form nanoparticles will be biologically characterized. The production and physico

chemical characterization of these self assembly systems will be performed by the two partnering institutions. TRC will be assessing the biological effects by studying the profile of antibodies and interaction with lymphocytes in HIV infected individuals reacting with these self assembly systems. During the period under review, the initial characterization of a system using cardamom mosaic virus coat protein has been carried out.

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STATISTICAL RESEARCH

An artificial neural network model for predicting TB

Background

Prediction models to identify patients with active TB have been lacking. The reason for this lies in the complexity of the clinical and radiographic presentation, the relatively small patient samples, and the use of modeling techniques that are poorly suited for the task. Previously, El-Solh *et al*. introduced a classification tree to assist physicians in their decision regarding whether respiratory isolation for suspicion of active pulmonary TB is needed. The model achieved a high degree of sensitivity at the expense of low specificity. Neural networks are computation systems that process information in parallel, using large numbers of simple units, and that excel in tasks involving pattern recognition. These intrinsic properties of the neural networks have been translated into higher performance accuracy in outcome prediction compared to expert opinion or conventional statistical methods.

Aim

- To build a neural network model for early prediction of pulmonaryTB

Methods

A total of 451 (195 culture positive, 256 culture negative) patients' data referred to the clinical trials of the Centre from January 2006 to March 2007 were considered for the artificial neural network **(**ANN) model. A set of 15 signs and symptoms were used for constructing the ANN model with back propagation error correction. An algorithm called the summation algorithm was developed based on the frequency of occurrence of the combination of signs and symptoms. A Perl code was written for implementing the summation algorithm. The resultant pattern specific outputs extracted by the Perl program were given as inputs into the back propagation network.

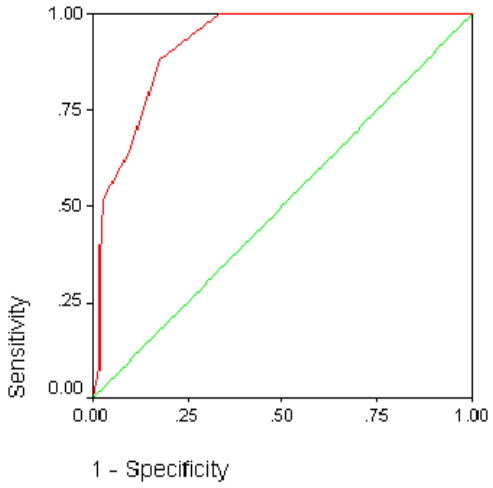
Results

A prediction accuracy of exact 91% was achieved. A root mean square error of 9% and a correlation of 0.97 were achieved between the predicted and observed results (Fig.29).

Conclusions

The ANN can helps physicians in a faster and efficient decision-making in isolating the TB patients based on the presented clinical symptoms. Fuzziness added to the input further increases the accuracy of prediction. Further work is continuing to improve the performance using other feature extraction techniques to improve accuracy.

Fig.29: ROC curve



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ELECTRONIC DATA PROCESSING

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

Data entry and data verification training was provided to a staff of JALMA, Agra for the “Prevalence of TB survey” during the month of March.

Data entry, information process and e-mailing are the key requirements for our research organization. These require a very secure and robust infrastructure. Much of the focus of the past year has been directed towards increasing the capacity of the existing IT infrastructure. Significant developments during the year include the up-gradation of network servers, completion of implementation of the new e-mail system of the staff, provision of a projector in each conference room and provision of a fast bandwidth link to the user desktop. We also completed replacing the network cabling throughout TRC and local hubs with switches and fiber uplinks to provide users with high bandwidth connection. The upgradation of the servers and other IT equipments that were undertaken in the previous year is being maintained during the current year.

This division helps in providing audio-visual system for the presentation of research materials during conferences, meetings and trainings that are regularly organised.

All breakdown calls of computers and its peripherals are dealt with under comprehensive annual maintenance contract. This includes managing the installation of facilities and ensuring that the computers are maintained and kept up to date.

The staff strength of this division comprises of four data entry/verification operators, five data processing assistants, one network coordinator (ICER project) and one division-in charge.

The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April 2007 to January 2008 is shown below.

No. of documents entered: 2,20,200

No. of documents verified: 2,21,588

A total of 1,21,952 records were processed for the on-going second and third resurveys of disease survey conducted at Tiruvallur district. Thirty two panchayats' pre-printed cards, and 23 panchayats' person's alphabetical name-wise and household-wise lists were supplied for the third resurvey. The second resurvey data clean work was completed. The data analyses were done and a report has been prepared and sent for publication.

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LIBRARY & INFORMATION SERVICES

The Library and information services of TRC, is a nodal agency to gather health information and make it accessible to the scientific community. The electronic resource services (ERS) enhances the customized integrated (24 hrs) access facility to our patrons. The e-journal village (http://www.trc-chennai.org/html/lis.html) provides our patrons with a single, comprehensive online list of titles to which we subscribe along with our web based value added services.

Collection building

Our collection building has been built with e-bundle, subject collection, cumulative collection, archives, databases and consortium (table 24).

Table 24: Print/e- Journals/Databases/ e-bundle/Subject Collection/Cumulative Collection/Archives

PRINT	Nos.
Indian Journals	7
International Journals	2
ELECTRONIC	
International Individual Journals	21
American Society for Microbiology (cumulative collection)	11
Annual Reviews Biomedical Suite (e-bundle with archives)	21
IndiaHealthStat (database)	1
MD Consult e-book	52
MD Consult Journals (database)	84
OVID (database)	2
Science Classic (archives)	424
ScienceDirect • Individual Titles • Current Opinion in & Trends in… titles (cumulative collection) • Immunology & Microbiology (subject collection)	 3 25 76
Total subscribed titles (Print 9 + e720)	729
Consortium (IP based) ERMED (http://www.nmlermed.in/index.htm) JCCC@ICMR (http://203.200.51.150/) J-Gate (Open Access Journals) http://www.j-gate.informindia.co.in/	 862 478 4440
Resource Sharing Proquest (database)	 975

Fig. 30: Subscribed resources

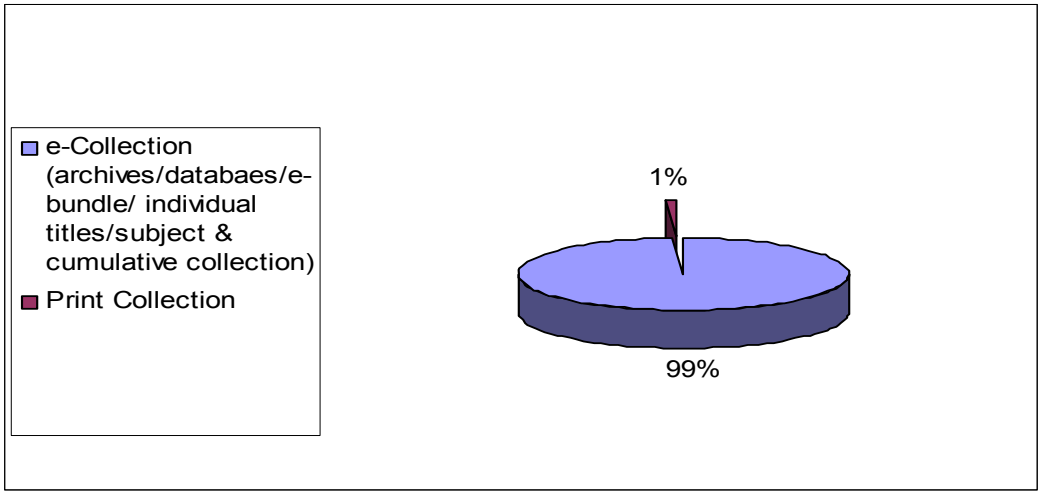
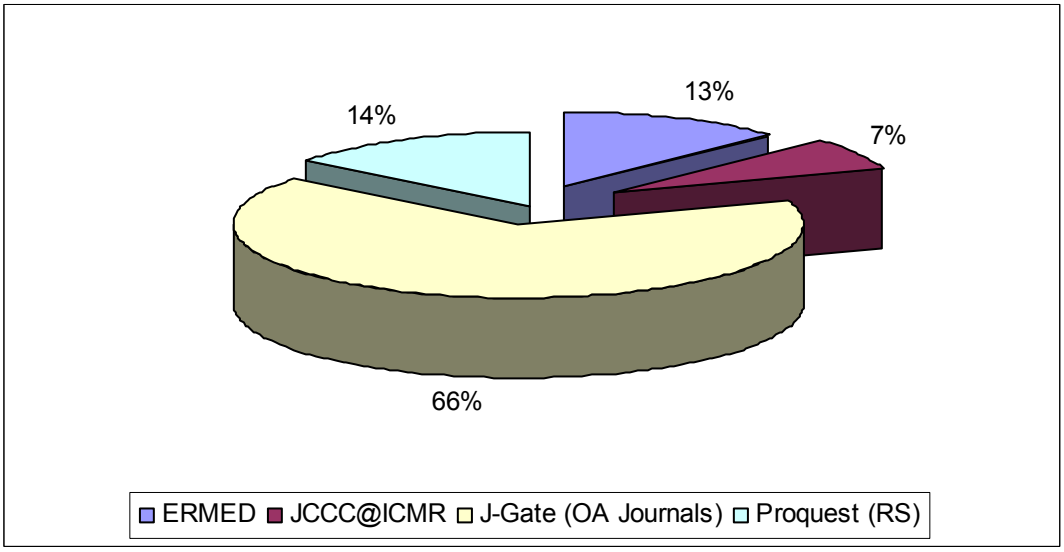


Fig. 31: Consortium/OA journals/Resource sharing



Multimedia Unit

The Multimedia Unit has designed the TRC web-site and uploaded recently, using html, photoshop, dreamweaver and flash software. Further its' services include Annual Report co-ordination for designing and compiling, co-ordination for scientific publications and thesis work relating to scanning and editing, and portable document format conversion.

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LIST OF PUBLICATIONS – 2007-08 (i.e. April 2007 to March 2008)

APPENDICES

Publications : 62

Publications in	i)	International Journals : 37
	ii)	National Journals : 25
Accepted for publication in	i)	International Journals : 19
	ii)	National Journals : 10

International:

1. Alagarasu K, Selvaraj P, Swaminathan S, Raghavan S, Narendran G, Narayanan PR. Mannose binding lectin gene variants and susceptibility to tuberculosis in HIV-1 infected patients of South India. Tuberculosis (Edinb).2007;87(6):535-543.

2. Angra P, Becx-Bleumink M, Gilpin C, Joloba M, Jost K, Kam KM, Kim SJ, Lumb R, Mitarai S, Ramsay A, Ridderhof J, Rieder HL, Selvakumar N, van Beers S, van Cleeff M, Van Deun A, Vincent V. Ziehl-Neelsen staining: strong red on weak blue, or weak red under strong blue? Int J Tuberc Lung Dis.2007; 11(11):1160–1161.

3. Bethunaickan R, Baulard AR, Locht C, Raja A. Antibody response in pulmonary tuberculosis against recombinant 27kDa (MPT51, Rv3803c) protein of *Mycobacterium tuberculosis*. Scand J Infect Dis.2007;39(10):867-874.

4. Flores L, Van T, Narayanan S, DeRiemer K, Kato-Maeda M, Gagneux S. Large sequence polymorphisms classify *Mycobacterium tuberculosis* strains with ancestral spoligotyping patterns. J Clin Microbiol.2007;45(10):3393–3395.

5. Gilpin C, Kim SJ, Lumb R, Rieder HL, Van Deun A, Working Group on Sputum Smear Microscopy. Critical appraisal of current recommendations and practices for tuberculosis sputum smear microscopy [Workshop Report]. Int J Tuberc Lung Dis.2007;11(9):946–952.

6. Gomathi NS, Sameer H, Kumar V, Balaji S, Dustackeer VN, Narayanan PR. *In silico* analysis of mycobacteriophage Che12 genome: characterization of genes required to lysogenise *Mycobacterium tuberculosis.* Comput Biol Chem.2007;31(2):82-91.

7. Harishankar M, Selvaraj P, Rajeswari DN, Anand SP, Narayanan PR. Promoter polymorphism of IL-18 gene in pulmonary tuberculosis in south Indian population. Int J Immunogenet.2007;34(5):317–320.

8. Hemanth Kumar AK, Ramachandran G, Kumar P, Anitha S, Rajasekaran S, Swaminathan S. Effect of anticoagulants on plasma nevirapine and efavirenz concentrations. Ther Drug Monit.2007;29(5):652-654.

9. Joseph MR, Shoby CT, Amma GR, Chauhan LS, Paramasivan CN. Surveillance of anti-tuberculosis drug resistance in Ernakulam Distrct, Kerala State, South India. Int J Tuberc Lung Dis.2007;11(4):443-449.

10. Kolappan C, Gopi PG, Subramani R, Narayanan PR. Selected biological and behavioural risk factors associated with pulmonary tuberculosis. Int J Tuberc Lung Dis.2007;11(9):999–1003.

11. Muniyandi M, Rajeswari R, Balasubramanian R, Nirupa C, Gopi PG, Jaggarajamma K, Sheela F, Narayanan PR. Evaluation of post-treatment health-related quality of life (HRQoL) among tuberculosis patients. Int J Tuberc Lung Dis.2007;11(8):887–892.

12. Muniyandi M, Ramachandran R, Gopi PG, Chandrasekaran V, Subramani R, Sadacharam K, Kumaran P, Santha T, Wares F, Narayanan PR. The prevalence of tuberculosis in different economic strata: a community survey from south India. Int J Tuberc Lung Dis.2007;11(9):1042–1045.

13. Murthy MK, Parasa RR, Deenadayalan A, Sharma P, Raja A. Evaluation of the diagnostic potential of region of deletion-1–encoded antigen culture filtrate protein-10 in pulmonary tuberculosis. Diagn Microbiol Infect Dis.2007;59(3):295–302.

14. Natarajan P, Narayanan S. *Mycobacterium tuberculosis* H37Rv induces monocytic release of interleukin-6 via activation of mitogen-activated protein kinases: inhibition by N-acetyl-l-cysteine. FEMS Immunol Med Microbiol.2007; 50(3):309-318.

15. Rajeswari DN, Selvaraj P, Raghavan S, Jawahar MS, Narayanan PR. Influence of HLA-DR2 on perforin-positive cells in pulmonary tuberculosis. Int J Immunogenet.2007;34(5):379–384.

16. Ramachandran G, Hemanthkumar AK, Rajasekaran S, Padmapriyadarsini C, Narendran G, Anitha S, Subramanyam S, Kumaraswami V, Swaminathan S. Steady-state pharmacokinetics of Nevirapine in HIV-1 infected adults in India. J Int Assoc Physicians AIDS Care.2007;6(4):251-254.

17. Ramachandran G, Hemanth Kumar AK, Sukumar B, Kumaraswami V, Swaminathan S. Single dose pharmacokinetics of Efavirenz in healthy Indian subjects. SAARC J Tuberc Lung Dis HIV/AIDS.2007;4(1):38-43.

18. Ramakrishnan K, Shenbagarathai R, Uma A, Kavitha K, Rajendran R, Thirumalaikolundusubramanian P. Prevalence of intestinal parasitic infestation in HIV/AIDS patients with diarrhea in Madurai City, South India. Jpn J Infect Dis 2007;60(4):209-210.

19. Rekha B, Swaminathan S. Childhood tuberculosis – global epidemiology and the impact of HIV. Paediatr Respir Rev.2007;8(2):99-106.

20. Ridderhof JC, van Deun A, Kam KM, Narayanan PR, Aziz MA. Roles of laboratories and laboratory systems in effective tuberculosis programmes. Bull World Health Organ.2007;85(5):354-359.

21. Selvakumar S, Narayanan S. Identification and characterization of the regulatory elements of the inducible acetamidase operon from *Mycobacterium smegmatis*. Can J Microbiol.2007;53(5):599-606.

22. Selvaraj P, Rajeswari DN, Jawahar MS, Narayanan PR. Influence of HLA-DRB1 alleles on Th1 and Th2 cytokine response to *Mycobacterium tuberculosis* antigens in pulmonary tuberculosis. Tuberculosis (Edinb).2007;87(6):544–550.

23. Shenoy RK, Suma TK, Kumaraswami V, Rahman N, Dhananjayan G, Padma S, Abhilash G, Ramesh C. Preliminary findings from a cross-sectional study on lymphatic filariasis in children, in an area of India endemic for *Brugia malayi* infection. Ann Trop Med Parasitol.2007;101(3):205-213.

24. Srimathi S, Jayaraman G, Feller G, Danielsson B, Narayanan PR. Intrinsic halotolerance of the psychrophilic α-amylase from *Pseudoalteromonas haloplanktis.* Extremophiles.2007;11(3):505-515.

25. Soundararajan L, Karunaianandham R, Jauvin V, Schrive MH, Ramachandran R, Narayanan PR, Fleury HJ, Swaminathan S. Characterization of HIV-1 isolates from antiretroviral drug-naive children in southern India. AIDS Res Hum Retroviruses.2007;23(9):1119-1126.

26. Subramani R, Santha T, Frieden TR, Radhakrishna S, Gopi PG, Selvakumar N, Sadacharam K, Narayanan PR. Active community surveillance of the impact of different tuberculosis control measures, Tiruvallur, South India, 1968-2001. Int J Epidemiol.2007;36(2):387-393.

27. Sulochana S, Narayanan S, Paramasivan CN, Suganthi C, Narayanan PR. Analysis of Fluoroquinolone resistance in clinical isolates of *Mycobacterium tuberculosis* from India. J Chemother.2007;19(2):166-171.

28. Swaminathan S, Padmapriyadarsini C, Ramachandran R, Menon PA, Mohan NS, Kubendiran G, Paramasivan CN. Localized pulmonary *Mycobacterium avium* disease in HIV-infected patients without access to HAART. J Int Assoc Physicians AIDS Care.2007;6(4):264-268.

29. Thomas BE, Ramachandran R, Anitha S, Swaminathan S. Feasibility of routine HIV testing among TB patients through a voluntary counselling and testing centre. Int J Tuberc Lung Dis.2007;11(12):1296–1301.

30. Vidyarani M, Selvaraj P, Jawahar MS, Narayanan PR. 1, 25 Dihydroxyvitamin D_3 modulated cytokine response in pulmonary tuberculosis. Cytokine.2007;40(2):128–134.

31. Vinothkumar TS, Kavitha S, Lakshminarayanan L, Gomathi NS, Kumar V. Influence of irrigating needle-tip designs in removing bacteria inoculated into instrumented root canals measured using single-tube luminometer. J Endod.2007;33(6):746-748.

32. Gopalaswamy R, Narayanan S, Jacobs WR Jr, Av-Gay Y. *Mycobacterium smegmatis* biofilm formation and sliding motility are affected by the serine/threonine protein kinase PknF. FEMS Microbiol Lett. 2008;278(1):121–127.

33. Jayakumar D, Jacobs WR Jr, Narayanan S. Protein kinase E of *Mycobacterium tuberculosis* has a role in the nitric oxide stress response and apoptosis in a human macrophage model of infection. Cell Microbiol.2008;10(2):365–374.

34. Kolappan C, Subramani R, Kumaraswami V, Santha T, Narayanan PR. Excess mortality and risk factors for mortality among a cohort of TB patients from rural south India. Int J Tuberc Lung Dis.2008;12(1):81–86.

35. Nusrath Unissa A, Selvakumar N, Narayanan S, Narayanan PR. Molecular analysis of isoniazid-resistant clinical isolates of *Mycobacterium tuberculosis* from India. Int J Antimicrob Agents.2008;31(1):71–75.

36. Swaminathan S, Subbaraman R, Venkatesan P, Subramanyam S, Kumar SR, Mayer KH, Narayanan PR. Tuberculin skin test results in HIV-infected patients in India: implications for latent tuberculosis treatment. Int J Tuberc Lung Dis. 2008;12(2):168–173.

37. Swaminathan S, Padmapriyadarsini C, Sukumar B, Iliyas S, Kumar SR, Triveni C, Gomathy P, Thomas B, Mathew M, Narayanan PR. Nutritional status of persons with HIV infection, persons with HIV infection and tuberculosis, and HIV-negative individuals from southern India. Clin Infect Dis.2008;46(6):946-949.

National:

1. Aravindhan V, Sulochana S, Narayanan S, Paramasivam CN, Narayanan PR. Identification & differentiation of *Mycobacterium avium* & *M. intracellulare* by PCR- RFLP assay using the *groES* gene. Indian J Med Res.2007;126(6):575-579.

2. Balasubramanian R, Ramanathan U, Thyagarajan K, Ramachandran R, Rajaram K, Bhaskar D, Shantharam, Hariharan RS, Narayanan PR. Evaluation of an intermittent six-month regimen in new pulmonary tuberculosis patients with diabetes mellitus. Indian J Tuberc.2007;54(4):168-176.

3. Ganga Devi NP, Rathinam SN, Ramachandran R, Swaminathan S. Recurrent herpes zoster in early childhood. Indian J Pediatr.2007;74(8):774-776.

4. Ganapathy S, Thomas BE, Jawahar MS, Josephine K, Sivasubramaniam, Weiss M. Perceptions of gender and tuberculosis in a south Indian urban community. Indian J Tuberc.2008;55(1):9-14.

5. Gopi PG, Vasantha M, Muniyandi M, Chandrasekaran V, Balasubramanian R, Narayanan PR. Risk factors for non-adherence to directly observed treatment (DOT) in a rural tuberculosis unit, south India. Indian J Tuberc.2007;54(2):66-70.

6. Gopi PG, Vasantha M, Kolappan C, Narayanan PR. Comparison of tuberculin reaction sizes at 48 and 72 hours among children in Tiruvallur district, south india. Indian J Tuberc.2007;54(3):152-156.

7. Gopi PG, Subramani R, Chandrasekaran V, Santha T, Narayanan PR. Impact of improved treatment success on the prevalence of TB in a rural community based on active surveillance. Indian J Tuberc.2008;55(1);22-27.

8. Jaggarajamma K, Sudha G, Chandrasekaran V, Nirupa C, Thomas A, Santha T, Muniyandi M, Narayanan PR. Reasons for non-compliance among patients treated under Revised National Tuberculosis Control Programme (RNTCP), Tiruvallur District, south India. Indian J Tuberc.2007;54(3):130-135.

9. Mathew S, Mohan NS, Rehman F, Dastageer A, Sundaram V, Paramasivan CN. Simple direct drug susceptibility tests on sputum samples for early detection of resistance in tubercle bacilli. Indian J Tuberc.2007;54(4):184-189.

10. Prabhu Anand S, Selvaraj P, Jawahar MS, Adhilakshmi AR, Narayanan PR. Interleukin-12B & interleukin-10 gene polymorphisms in pulmonary tuberculosis. Indian J Med Res.2007;126(2):135-138.

11. Raghu B, Venkatesan P. Effect of n-3 fatty acid supplementation on blood glucose, lipid profile and cytokines in humans: a pilot study. Indian J Clin Biochem.2008;23(1):85-88.

12. Ramachandran G, Hemanth Kumar AK, Sarala K, Padmapriyadarsini C, Anitha S, Tharani CB, Kumaraswami V, Swaminathan S. Urine levels of rifampicin & isoniazid in asymptomatic HIV-positive individuals. Indian J Med Res.2007;125(6):763-766.

13. Rekha VV, Balasubramanian R, Swaminathan S, Ramachandran R, Rehman F, Sundaram V, Thyagarajan K, Selvakumar N, Adhilakshmi AR, Iliayas S, Narayanan PR. Sputum conversion at the end of intensive phase of Category-1 regimen in the treatment of pulmonary tuberculosis patients with diabetes mellitus or HIV infection: An analysis of risk factors. Indian J Med Res. 2007;126(5):452-458.

14. Sadacharam K, Gopi PG, Chandrasekaran V, Eusuff SI, Subramani R, Santha T, Narayanan PR. Status of smear-positive TB patients at 2-3 years after initiation of treatment under a DOTS programme. Indian J Tuberc.2007;54(4):199-203.

15. Swaminathan S, Narendran G, Menon PA, Padmapriyadarsini C, Arunkumar N, Sudharshanam NM, Ramesh Kumar S, Chandrasekhar S. Impact of HIV infection on radiographic features in patients with pulmonary tuberculosis. Indian J Chest Dis Allied Sci.2007;49(3):133-136.

16. Swaminathan S, Padmapriyadarsini C, Ponnuraja C, Sumathi CH, Rajasekaran S, Amerandran VA, Reddy MVK, Deivanayagam CN. Miliary tuberculosis in human immunodeficiency virus infected patients not on antiretroviral therapy: Clinical profile and response to short-course chemotherapy. J Postgrad Med.2007;53(4):228-231.

17. Swaminathan S, Deivanayagam CN, Rajasekaran S, Venkatesan P, Padmapriyadarsini C, Menon PA, Ponnuraja C, Dilip M. Long term follow up of HIV-infected patients with tuberculosis treated with 6-month

intermittent short course chemotherapy. Natl Med J India.2008;21(1):3-8.

18. Thomas A, Ramachandran R, Rehman F, Jaggarajamma K, Santha T, Selvakumar N, Krishnan N, Mohan NS, Sundaram V, Wares F, Narayanan PR. Management of multi drug resistant tuberculosis in the field:Tuberculosis Research Centre experience. Indian J Tuberc.2007;54(3):117-124.

19. Tuberculosis Research Centre. Evaluation of a non-rifampicin continuation phase (6HE) following thrice-weekly intensive phase for the treatment of new sputum positive pulmonary tuberculosis. Indian J Tuberc.2007;54(2):84-90.

20. Tuberculosis Research Centre. Limitations of conducting community surveys to assess the epidemiological impact of TB control programmes on the incidence of TB. Indian J Tuberc.2008;55(1):1-4.

21. Vadivel J, Kripasankar, Gunasundari A. Knowledge, attitude on tuberculosis among nursing students. Nurses of India.2007;9-11.

22. Vallinayagam V, Senthamaraikannan K, Venkatesan P. A Markov chain Monte Carlo algorithm for multiple imputation of missing values. Book published by Allied Publishers P Ltd. on Mathematics, Computing and Modeling. Ed. P. Balasubramaniam and R. Uthayakumar. 2007;257-264.

23. Venkatesan P, Suresh ML. Neural network model for classifying renal failure. Book published by Allied Publishers P Ltd. on Mathematics, Computing and Modeling. Ed. P. Balasubramaniam and R. Uthayakumar. 2007;155-160.

24. Venkatesan P, Porchelvan S. AIDS projection using non-parametric back-calculation method. Book published by Allied Publishers P Ltd. on Mathematics, Computing and Modeling. Ed. P. Balasubramaniam and R. Uthayakumar. 2007;283-288.

25. Vidyarani M, Selvaraj P, Jawahar MS, Rajeswari DN, Anbalagan S, Narayanan PR. Intracellular granzyme A expression of peripheral blood lymphocyte subsets in pulmonary tuberculosis. Curr Sci.2007;93(6):823-825.

Accepted for publication:

International:

1. Bakshi S, Ramachandran G, Ramesh K, Hemanthkumar AK, Anitha S, Padmapriyadarsini C, Narendran G, Menon PA, Rajasekaran S, Swaminathan S. Study of *ABCB1* polymorphism (C3435T) in HIV-1-infected individuals from south India. Br J Clin Pharmacol.

2. Dusthackeer A, Kumar V, Selvakumar S, Sivaramakrishnan G, Zhu G, Subramanyam B, Hassan S, Selvakumar N, Chan J, Narayanan PR. Construction and evaluation of luciferase reporter phages for the detection of non-replicating tubercle bacilli. J Microbiol Methods.

3. Dusthackeer A, Hassan S, Kumar V. Tape measure protein having MT3 motif facilitates phage entry into stationary phase cells of *Mycobacterium tuberculosis*. Comput Biol Chem.

4. Gosoniu GD, Ganapathy S, Kemp J, Auer C, Somma D, Karim F, Weiss MG. Gender and socio-cultural determinants of delay to diagnosis of TB in Bangladesh, India and Malawi. Int J Tuberc Lung Dis.

5. Gupta RN, Wyatt GE, Swaminathan S, Rewari BB, Locke TF, Ranganath V, Sumner LA, Liu H. Correlates of relationship, psychological, and sexual behavioral factors for HIV risk among Indian women. Cultur Divers Ethnic Minor Psychol.

6. Kumar V, Loganathan P, Sivaramakrishnan G, Kriakov J, Dusthackeer A, Subramanyam B, Chan J, Jacobs WR Jr, Narayanan PR. Characterization of temperate phage Che12 and construction of a new tool for diagnosis of tuberculosis. Tuberculosis.

7. Lakshminarayan H, Narayanan S, Bach H, Sundaram KG, Av-Gay Y. Molecular cloning and biochemical characterization of a serine threonine protein kinase, PknL, from *Mycobacterium tuberculosis*. Protein Expr Purif.

8. Narayanan S, Gagneux S, Hari L, Tsolaki AG, Rajasekhar S, Narayanan PR, Small PM, Holmes S, Deriemer K. Genomic interrogation of ancestral *Mycobacterium tuberculosis* from south India. Infect Genet Evol.

9. Pokkali S, Das SD, Logamurthy R. Expression of CXC and CC type of chemokines and its receptors in tuberculous and non-tuberculous effusions. Cytokine.

10. Prabha C, Rajashree P, Das SD. TLR2 and TLR4 expression on the immune cells of tuberculous pleural fluid. Immunol Lett.

11. Raja A, Ranganathan UD, Bethunaickan R. Improved diagnosis of pulmonary tuberculosis by detection of antibodies against multiple *Mycobacterium tuberculosis* antigens. Diagn Microbiol Infect Dis.

12. Rajashree P, Supriya P, Das SD. Differential migration of human monocyte-derived dendritic cells after infection with prevalent clinical strains of *Mycobacterium tuberculosis*. Immunobiology.

13. Rajashree P, Das SD. Infection with prevalent clinical strains of *Mycobacterium tuberculosis* leads to differential maturation of monocyte derived dendritic cells. Immunol Lett.

14. Rajashree P, Krishnan G, Das SD. Impaired phenotype and function of monocyte derived dendritic cells in pulmonary tuberculosis. Tuberculosis.

15. Ramana Rao PV, Rajasekaran S, Raja A. Augumentation of natural Killer activity with exogenous interleukins in patients with HIV and pulmonary tuberculosis co-infection. AIDS Res Hum Retroviruses.

16. Ramakrishnan K, Shenbagarathai R, Kavitha K, Uma A, Balasubramaniam R, Thirumalaikolundusubramanian P. Serum zinc and albumin levels in pulmonary tuberculosis patients with and without HIV. Jpn J Infect Dis.

17. Selvaraj P, Alagarasu K, Harishankar M, Vidyarani M, Rajeswari DN, Narayanan PR. Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis. Cytokine.

18. Selvaraj P, Alagarasu K, Harishankar M, Vidyarani M, Narayanan PR. Regulatory region polymorphisms of Vitamin D receptor gene in pulmonary tuberculosis patients and normal healthy subjects of south India. Int J Immunogenetics.

19. Selvaraj P, Vidyarani M, Alagarasu K, Prabhu Anand S, Narayanan PR. Regulatory role of promoter and 3' UTR variants of Vitamin D receptor gene on cytokine response in pulmonary tuberculosis. J Clin Immunol.

20. Senbagavalli R, Geetha ST, Karunakaran K, Banu Rekha VV, Venkatesan P, Ramanathan VD. Reduced erythrocyte CR1 levels in patients with pulmonary tuberculosis is an acquired phenomenon. Clin Immunol.

21. Somma D, Thomas BE, Karim F, Kemp J, Arias N, Auer C, Gosoniu GD, Abouihia A, Weiss MG. Gender and socio-cultural determinants of TB-related stigma in Bangladesh, India, Malawi and Colombia. Int J Tuberc Lung Dis.

22. Supriya P, Prabha C, Das SD. Diagnostic utility of interferon-γ induced protein of 10kDa (IP-10) in tuberculous pleurisy. Diagn Microbiol Infect Dis.

23. Swaminathan S, Hanna LE, Sundaramurthi JC, Leonard A, Angayarkanni B, Francis AC, Lakshmi S, Nayak K. Prevalence and pattern of cross-reacting antibodies to HIV in patients with tuberculosis. AIDS Res Hum Retroviruses.

24. Weiss MG, Somma D, Karim F, Abouihia A, Auer C, Kemp J, Jawahar MS. Cultural epidemiology of TB with reference to gender in Bangladesh, India and Malawi. Int J Tuberc Lung Dis.

National

1. Ganga Devi N, Shenbagavalli R, Subramanyam S, Subramani K, Ramesh K, Rathinam SN, Swaminathan S. Rapid progression of HIV infection in infancy. Indian Pediatr.

2. Prabha C, Supriya P, Das SD, Sukumar B, Balaji S. Leptin response in patients with tuberculous pleuritis. Indian J Med Res.

3. Rajavelu P, Das SD. Expression of co-stimulatory molecules B7.1 & B7.2 on macrophages infected with various strains of *Mycobacterium tuberculosis* & its influence on T-cell apoptosis. Indian J Med Res.

4. Swaminathan S, Datta M, Radhamani MP, Mathew S, Reetha AM, Rajajee S, Mathew R, Radhakrishnan A, Raghu MB. A profile of bacteriologically confirmed pulmonary tuberculosis in children. Indian Pediatr.

5. Swaminathan S, Antony L, Venkatesan P, Hanna LE, Angayarkanni B, Ponnuraja C, Robin J, Precilla KL, Ramachandran R. Sensitivity and specificity of combination testing algorithms for human immunodeficiency virus (HIV) in a tuberculosis clinic. Indian J Med Res.

6. Vasantha M, Gopi PG, Subramani R. Survival of tuberculosis patients treated under DOTS in a rural tuberculosis unit (TU), south India. Indian J Tuberc.

7. Thomas A, Chandrasekaran V, Joseph P, Rao VB, Patil AB, Jain DK, Chowdhary D, Saibabu, Mahapatra S, Devi S, Wares F, Narayanan PR. Increased yield of smear positive pulmonary TB cases by screening patients with $\geq$2 weeks cough, compared to $\geq$3 weeks and adequacy of 2 sputum smear examinations for diagnosis. Indian J Tuberc.

8. Venkatesan P. Markov chain Monte Carlo methods in Bayesian inference. In: Applied Bayesian Stat. Analysis.

9. Venkatesan P, Srinivasan R. Bayesian models for HIV-AIDS in India. A spatial analysis. In: Applied Bayesian Stat. Analysis.

10. Venkatesan P, Ponnuraja C. Bayesian separate and joint modeling for controlled clinical trial data using BUGS. In: Applied Bayesian Stat. Analysis.

Conferences / Workshops /training programs attended

1. Address on HIV/AIDS update held at Pune during April 2007 – Soumya Swaminathan.

2. Training on 3100 Avant Genetic Analyzer held at New Delhi during April 2007 - K Ramesh & S Lakshmi.

3. Workshop on Right to Information Act held at Institute of Secretariat Training & Management, Jodhpur during April 2007- P Selvaraj.

4. Workshop on Right to Information Act held at Bangalore during May 2007 – P Selvaraj.

5. Workshop on Fundamentals of Biostatistics in Clinical Research held at New Delhi during May 2007 – V V Banu Rekha.

6. Short Course in Biostatistics held at Vellore during June 2007 - G Narendran.

7. Training on the theoretical and practical aspects of Liquid Chromatography Mass Spectrometry held at Mumbai during June 2007 - Geetha Ramachandran &.Hemanth Kumar

8. Workshop on developing a protocol for a Cochrane Systematic Review held at Vellore during June 2007 - V V Banu Rekha.

9. Expert Group meeting of REP during June 2007 - Beena E Thomas

10. Guest lecture at the symposium to enhance HIV-AIDS research capacity held at Bangalore during July 2007 - Soumya Swaminathan.

11. Steering Committee meeting on Nutrition and HIV/AIDS held at Vienna, Austria during July 2007 - Soumya Swaminthan.

12. Guest lecture at the IAS Conference held at Sydney, Australia during July 2007 – Soumya Swaminthan.

13. Paper presented at the Polytechnic University, Hong Kong during July 2007 - Beena E Thomas.

14. AIDS workshop at JNCASR, Bangalore during July 2007 – Sujatha Narayanan.

15. Fourth Convention of the National Alliance for Mission Centre held at IGNOU, New Delhi during August 2007 - Soumya Swaminathan.

16. Guest lecture at the 8th International Congress on AIDS in Asia & Pacific held at Colombo, Srilanka during August 2007 - Soumya Swaminathan.

17. Training Program for ICMR Scientists on Bioinformatics at Institute of Bioinformatics and Applied Biotechnology, Bangalore during August 2007 – Jagadish Chandrabose and Sameer Hassan.

18. Guest lecture at American College of Chest Physicians held at Chennai - August 2007 – M S Jawahar.

19. Sccientific writing workshop held at Chennai during Aug 2007 – Hemanth Kumar, G Narendran, M Vasantha, Mohanarani Suhadev & S Ramesh Kumar.

20. Training in Clinical pharmacology of antiretroviral drugs under Fogarty AITRP at the Tufts University School of Medicine, Boston during Aug – Dec 2007 – Geetha Ramachandran.

21. National Conference on Biotechnology and Bioinformatics in Pursuit of Excellence and Relevance for Human Kind, held at Chennai, during September 2007- Jagadish Chandrabose.

22. A Paradigm Shift in the Drug Designing held at SRM University, Chennai during September 2007 - Jagadish Chandrabose.

23. Consultative Meeting on MDR/XDR Tuberculosis held at TRC, Chennai during September 2007 - M S Jawahar.

24. Guest lecture on Multi Drug Resistant Tuberculosis held at Meenakshi Medical College, Kancheepuram during September 2007 - M S Jawahar.

25. Clinical and Translational Research for Prevention, Care and Treatment of HIV – related Co-Morbidities at ICMR, New Delhi during September 2007 - Beena E Thomas.

26. Training workshop in Survey Research Methodology: Application and Challenges held at Vadodara during September 2007 - K Jaggarajamma, Mohanarani Suhadev, Rajasakthivel & P Murugesan.

27. Lecture on “Nutritional Considerations and Opportunities in ART and TB Treatment Programmes” held at Bangkok, Thailand during October 2007 – Soumya Swaminathan.

28. Symposium on Molecular modeling at Bharath University, Chennai during October 2007 – Sameer Hassan.

29. Lecture on proteomics at AU-KBC-MIT, Anna University, Chennai during October, 2007 - Sameer Hassan.

30. Conference on Contemporary issues and future challenges in Drug Development on New Drugs held at Mumbai during October 2007 - M S Jawhar.

31. Impact assessment meeting held at NTI, Bangalore, during October 2007 – R Subramani.

32. Guest lecture on Newer modalities of diagnosis for tuberculosis held at New Delhi during November 2007 – M S Jawahar.

33. Guest lecture on Diagnosis of TB in the Symposium on Tuberculosis Management held at Ahmedabad on November 2007 - Soumya Swaminthan.

34. Guest lecture on HIV drug resistance and its implications for treatment in India held at New Delhi during November 2007 – Soumya Swaminathan.

35. International Symposium on Obsessive-Compulsive Disorder (OCD) held at Bangalore during November 2007 - Pradeep A Menon.

36. Training course on Real-Time PCR held at New Delhi during November 2007 - P Selvaraj.

37. Training at Colorado State University Bioinformatics approaches for tuberculosis during November 2007 - Alamelu Raja.

38. Meeting on Prevalence Survey in Jabalpur District (new proposal) held at RMRCT, Jabalpur during November 2007 – R Subramani.

39. 25th Biennial Conference of Indian Association of Leprologists at Kanpur, November 2007 – Sujatha Narayanan.

40. Annual Symposium of Society of Biological Chemistry at University of Tirupathi, November 2007 – Sujatha Narayanan.

41. Guest Lecture on AIDS - Emerging and Re-Emerging of Viral Diseases held at New Delhi during December 2007 - Soumya Swaminathan.

42. Fundamentals of Biostatistics, Principles of Epidemiology & Statistical Package for Social Sciences held at C M C, Vellore during December 2007 - G Narendran.

43. 34th Indian Immunology Society Conference held at NARI, Pune during December 2007 – Alamelu Raja, Sujatha Narayanan, P Selvaraj, K Alagarasu, S Raghavan, S Anbalagan, Kaustuv Nayak, Harishankar M, Prabhu Anand S, Anbarasu D, M Madhan Kumar, Ramana Rao PV, Basirudeen S, Supriya P, P Rajashree.

44. Training on HIV care and treatment held at Chennai during December 2007 - Beena E Thomas.

45. 62nd National conference on TB and chest diseases held at New Delhi during December 2007 - K. Jaggarajamma.

46. Guest Lecture on Nutrition & Health held at Visakhapatnam during January 2008 – Soumya Swaminathan.

47. 60th Annual National Conference of Indian Psychiatric Society held at Kolkata during January 2008 – Pradeep A Menon.

48. Chennai ART Symposium – CART 2008 held at Chennai during January 2008 – P.K.Bhavani.

49. Global Trends in Biomedical Informatics: Research, Education and Commercialization held at Chennai during January 2008 – Luke Elizabeth Hanna.

50. Symposium on Chemical Biology: Its Impact on Drug Discovery and Development held at Hyderabad, during January 2008 - Jagadish Chandrabose, Sameer Hassan, K. Lucia Precilla & S Lakshmi.

51. Conference on Retroviruses and Opportunistic Infections (CROI 2008) held at Boston, USA during February 2008 – Soumya Swaminathan.

52. Statistical workshop on Randomized clinical trial designs and Outcome held by ICER at Chennai during February 2008 - Padmapriyadarsini, Bhavani.

53. Microbicides 2008 Conference organized by NIH and ICMR at New Delhi during February 2008 – C. Padmapriyadarsini, P.K. Bhavani, P Murugesan.

54. Symposium on Emerging Infectious Diseases, held at Chennai during February 2008 - M S Jawahar.

55. Alumni Association meeting held at Madras School of Social Work, Chennai during February 2008 –Beena E. Thomas.

56. Awareness Programme on Tuberculosis held at Chennai during February 2008 – Niruparani Charles, P Murugesan & A. Dhanalakshmi.

57. Training on Ethical Issues in Biomedical Research held at Chennai during February 2008 - Beena E Thomas, Niruparani Charles, K J Jagannatha Rao & Mohanarani Suhadev.

58. Survey Meeting on high risk groups organized by IBBA at TNSACS, Chennai during February 2008 – Beena E Thomas.

59. Mega Comprehensive Medical Camp held at Chennai during February 2008 – P Murugesan & A Dhanalakshmi.

60. Guest lecture on Shortening TB chemotherapy held at New Delhi March 2008 – M S Jawahar.

61. Workshop on Randomised Clinical Trials held at Bangalore, during March 2008 - M S Jawahar.

62. Guest Lecture on World TB Day Progamme held at Chennai during March 2008 – M S Jawahar.

63. Training programme on addressing concerns of women living with HIV in accessing schemes and policies in Chennai – during March 2008 - Beena E Thomas.

64. Lecture on Socio-behavioral Research of TRC, ICMR held at Chennai during March 2008 – Beena E Thomas.

65. "Is there a 3rd gender" meeting held at YWCA, Chennai during March 2008 - Beena E Thomas.

66. Training programme on Basic information on TB and associated with HIV and related problems for the community during March 2008 - Beena E Thomas.

67. Redesign and planning meeting for conducting BSS Wave XII held at Chennai during March 2008 – Beena E Thomas.

Special Assignments

Dr. Alamelu Raja

1. Expert Member of the Institutional Review Board of Sri Kanchi Kamakoti CHILDS Trust Hospital
2. Member, Editorial Board, Indian Journal of Medical Research.

3. Reviewer for journals:
   International:
   i. BMC Infectious Diseases
   ii. European Journal of Paediatrics
   iii. Journal of Tuberculosis and Lung Diseases

   National:
   i. Indian Journal of Medical Research
   ii. Indian Journal of Tuberculosis
   iii. Journal of Biochemistry and Biophysics
   iv. Reviewer of project proposals submitted to ICMR
   v. Member of Doctoral Committee for Ph. D. students for University of Madras.

Dr. Sujatha Narayanan

1. Examiner for Ph.D. thesis of –

   i. Jadavpur University, Kolkata
   ii. University of Kerala, RGCB, Trivandrum
   iii. M.Sc., (Molecular Biology), Stella Maris College, Chennai

2. Member - Madras University Selection Committee

Dr.P.Selvaraj

<u>Assignments related to research activities</u>:

1. Recognized Guide/Supervisor – Madras University, Chennai for guiding research work of candidates leading to Ph.D. degree
2. Executive council member, Indian society for histocompatibility and Immunogenetics, New Delhi
3. Reviewer for journals:
   International:

   i. American Journal of Respiratory & Critical Care Medicine
   ii. Scandinavian Journal of Immunology
   iii. BMC Bioinformatics
   iv. Human Immunology

v. Journal of Leukocyte Biology
vi. Applied Biochemistry & Biotechnology
vii. Human Genetics
viii. Cytokine

National:
i. Indian journal of experimental biology

4. External examiner for MSc, theory and practical exams for –
M.Sc., Human Genetics & Biotechnology
Sri Ramachandra Medical College and Research Institute, Chennai -116

Dr.D. Sulochana

1. Doctoral committee member for two Ph.D. students from the Department of Zoology, Biochemistry, University of Madras, Chennai
2. Viva Voce examiner for a Ph D student of Department of Zoology, University of Madras, Chennai
3. Reviewer for the Project from Cancer research UK funding agency
4. Reviewer for journals:
International:
i. The Journal of Infectious Diseases
ii. Human Immunology
iii. Molecular &Cellular Biochemistry
iv. Mediators of Inflammation

Dr.Geetha Ramachandran

1. Resource person for the HIV fellowship program held at YRG Care, Chennai.
2. Invited as judge for the Science Exhibition organized by the Department of Biochemistry, Ethiraj College, Chennai.
3. Reviewer of Doctoral Thesis of Post graduate Institute of Medicine, Chandigarh.
4. Reviewer for journals: Journal of Chromatographic Sciences, Indian Journal of Pharmacology, Indian Journal of Tuberculosis
5. Reviewer of research proposals submitted to ICMR.
6. Project Guide for MD (Pharmacology), M.Pharm., B.Tech (Biotechnology), M.Sc., (Biotechnology) & M.Phil (Bio Chemistry).

Dr. A.K.Hemanth Kumar:

1. Invited as judge for the Science Exhibition organized by the Department of Biochemistry, Ethiraj College, Chennai.

Dr.P. Venkatesan

1. Chairman - Institute Ethics Committee/Institute Review Board, Sri Ramachandra Medical University, Chennai
2. Chairman - Board of Studies - MSc (Bioinformatics) - Sri Ramachandra Medical University, Chennai
3. Chairman - Board of Studies - Batch (Medical Informatics) - Sri Ramachandra Medical University, Chennai
4. Expert Member - Scientific Advisory Board, Sai's Biosciences Research Institute, Chennai
5. Member- Board of Studies - MSc (Statistics) & M.Sc (Biostatistics), University of Madras, Chennai
6. Member - Board of Studies - MSc (Mathematics), & M.Phil (Mathematics), Periyar University, Salem
7. Member - Board of Studies - MSc (Human Genetics), Sri Ramachandra Medical University, Chennai
8. Member - Board of Studies - MTech (Bioinformatics) and BTech (Bioinformatics), Sathyabama University, Chennai
9. External Examiner-University of Madras, Tamilnadu Dr.MGR Medical University, & Sri Ramachandra University, Chennai
10. Member - Editorial Board: Journal of Pure and Applied Spectrophysics
11. General Secretary - Indian Society for Medical Statistics
12. Member - Executive Committee: International Biometric Society

Ph.D. Scholars

List of staff / students who have got their Ph.D. degree at the University of Madras

Sl. No.	Name of the candidates	Title of the Ph.D. degree	Supervisor/Guide
1.	Ms.R.Priya	Apoptosis of human monocytes and macrophages by *M.tuberculosis* and its implications on cell mediated immune response	D.Sulochana Das
2.	Ms.G.Senbagavalli	Serum and tissue complement profile in tuberculosis	Dr.V.D.Ramanathan
3.	Mr.V.Narayana Rao	Complement activation by strains of mycobacteria wild type and gene disrupted *M.tuberculosis* and recombinant BCG	Dr.V.D.Ramanathan
4.	Ms.M.Vidya Rani	Regulatory role of vitamin D receptor gene polymorphism on cytokine response in pulmonary tuberculosis	Dr.P.Selvaraj
5.	Ms.C.Prabha	Immune response in tuberculosis:Th1/Th2 paradigm	Dr.Sulochana Das
6.	Ms.P.Supriya	Role of chemokines in tuberculosis immunity	Dr.Sulochana Das
7.	Ms.Gomathi Sekar	Optimizing sputum microscopy to detect AFB	Dr.N.Selva Kumar
8.	Ms. Nisha Rajeswari	Influence of HLA-DR antigens on immune functions in pulmonary tuberculosis	Dr. P. Selvaraj

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

Sl.No.	Name of the candidate	Title of the Ph.D. degree	Supervisor/Guide
1.	Mr.S.Manivannan	The role of complement activation and antibody in the early interaction of *M.tuberculosis* and macrophages	Dr.V .D.Ramanathan
3.	Dr. P.L. Natarajan	Cellular immunology of TB and HIV/TB	Dr. Sujatha Narayanan
4.	Ms. Mohanarani Suhadev	Sociological aspects of HIV/AIDS	Dr. Udaya Mahadevan
5.	Mr.C.Ponnuraja	Frailty models	Dr.P.Venkatesan

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

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. project	Supervisor/Guide
1.	Mr. D. Anbarasu	CSIR	Identification and characterization of immunoreactive T-cell antigens of *M.tuberculosis*	Dr. Alamelu Raja
2	Mr. P.V. Ramana Rao	ICMR	Innate immunity in HIV infection	Dr. Alamelu Raja
3.	Mr. M. Madhan Kumar	CSIR	Cytotoxic cellular response in tuberculosis	Dr. Alamelu Raja
4.	Mr. S. Basirudeen	ICMR	Interferon gamma assay for latent TB infection in HIV patients	Dr. Alamelu Raja
5.	Ms.S. Lakshmi	ICMR	HIV drug resistance	Dr.P.R.Narayanan
6.	Mr. Kaustuv Nayak	ICMR	Evaluation of cellular immune response to infection with HIV-I C subtype in South India	Dr.P.R.Narayanan
7.	Ms.A. Nusrath Unissa	ICMR	Molecular studies on isoniazid resistance in *M.tuberculosis*	Dr.N. Selvakumar
8.	Mr.K. Alagarasu	ICMR	Gene polymorphism studies on chemokine, chemokine receptors, vitamin D Receptor and mannose binding lectin gene in HIV and HIV-TB patients	Dr.P. Selvaraj
9.	Mr.S.Raghavan	ICMR	Human Leucocycte Antigen polymorphism studies in HIV and HIV-TB patients	Dr.P. Selvaraj
10.	Mr.S. Prabhu Anand	CSIR	Regulatory effects of vitamin D_3 and vitamin D Receptor genotypes on VDR expression and cytokine production in pulmonary tuberculosis	Dr.P. Selvaraj
11.	Ms. Harini Laxminarayan	UGC	Study on molecular biology of *M. tuberculosis*	Dr. Sujatha Narayanan
12.	Ms. Aparna J Christy	ICMR	Development of epitope delivery system for construction of recombinant BCG vaccine for tuberculosis	Dr. Sujatha Narayanan
13.	Ms. V. Malini	ICMR	Functional characterization of FtsY, a signal recognition particle receptor from *M.tuberculosis*	Dr. Sujatha Narayanan
19.	Ms. N. Yamuna	UGC	Classification and regression trees	Dr. P. Venkatesan

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. project	Supervisor/Guide
20.	Ms. Neema Bourai	CSIR	Functional characterization of serine/threonine protein kinase 1 of *M.tuberculosis*	Dr. Sujatha Narayanan
21.	Mr.P. Dinesh	ICMR	A molecular approach to pathogenesis role of serine / threonine kinase PknE in signal transduction involved in host pathogen interactions	Dr. Sujatha Narayanan
22.	Ms.P. Rajashree	ICMR	Role of dendritic cells in tuberculous immunity	Dr. Sulochana Das
23.	Mr.S. Balaji	ICMR	Rapid diagnosis of *M. tuberculosis*	Dr. Vanaja Kumar
24.	Mr.M. Radhakrishnan	DST	Anti-TB drugs from actinomycetes	Dr. Vanaja Kumar
25.	Ms.R. Lakshmi	ICMR	Molecular studies on mycobacteria	Dr. Vanaja Kumar

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

Sl.No.	Name of the staff	Title of the Ph.D. degree	Supervisor/Guide
1	Ms. Nalini Sundar Mohan	Measurement of drug resistance in tuberculosis	Dr.C.N. Paramasivan
2	Ms.N.S. Gomathi	Rapid diagnosis and drug susceptibility testing of *M.tuberculosis*	Dr. Vanaja Kumar
3	Mr.V.N. Azgar Dusthackeer	Mycobacterial latency and tuberculosis diagnosis	Dr. Vanaja Kumar
4	Mr. Sameer Hassan	Genome analysis of phages and viruses	Dr. Vanaja Kumar
5	Mr. N. Arunkumar	Causal analysis	Dr.P. Venkatesan
6	Mr. B. Sukumar	Statistical methods for microarray data analysis	Dr.P. Venkatesan
7	Mr.Jagadish Chandra Bose	Immudominant epitopes against HIV subtype C	Dr. Luke Elizabeth Hanna
8	Mr. S Anbalagan	Innate and adaptive immunity in HIV	Dr. Luke Elizabeth Hanna
8	Mr. M Harishankar	Role of vitamin D receptor promoter & 3'UTR gene variants on Vitamin D modulated immune functions in tuberculosis	Dr.P. Selvaraj
8	Mr.S Sivakumar	Molecular epidemiology of tuberculosis	Dr. Sujatha Narayanan
8	Mr.R Srinivasan	Spatial analysis	Dr.P. Venkatesan
9	Mr.L Sekar	Survival analysis	Dr.P. Venkatesan
10	Dr. Ranjani Ramachandran	HIV associated opportunistic infections	Dr.C.N. Paramasivan

Research Associate:

Dr. S. Prabu Seenivasan is a Research Associate in the CSIR-funded project titled "Isolation of active compound(s) from selected medicinal plants against *M. tuberculosis*" under the supervision of Dr. Vanaja Kumar, Scientist "F" of Bacteriology Department.

Scientific / Technical / Administrative Staff

Director

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P.R. Narayanan, Ph.D., D.I.I.Sc.
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TRC

Scientist 'F'

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V.Kumaraswami, M.D., M.N.A.M.S., Ph.D.(Med.)
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M.S.Jawahar, M.D.,M.Sc.,D.L.S.H.T.M.
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Soumya Swaminathan, M.D., Dip.N.B (Paed).
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