Tuberculosis Research Centre, Chennai

Annual Report 2006- 07

WHO Collaborating Centre for Tuberculosis Research & Training

TUBERCULOSIS RESEARCH CENTRE

Annual Report

2006-2007

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PREFACE

Following pages represent the output of the scientific activities of TRC during the reporting period. I am grateful to all the scientific, technical, administrative and service staff for their support in generating high quality research at TRC. I congratulate the staff responsible for drafting this excellent report. If there are mistakes either in content, or in style, or in language I am responsible for the same. Please review this critically and provide your valuable comments and criticisms to improve the scientific output of this centre more and more in the forthcoming years.

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Name	Remarks
Prof. R. C. Mahajan J.N.Bose National Research Program Advisor, ECD-ICMR, & Emeritus Professor, PGI Chandigarh	*"Highly impressed with kind of infrastructure created in TRC and quality of work being carried out"*
Dr. Mel Spigelman Global Alliance for TB Drug Development USA	*"Wonderful facility and even more wonderful staff"*
Dr. Ann Ginsberg Global Alliance for TB Drug Development USA	*"Looking forward to working together on our mutual goal of improving TB treatment"*
Dr. Said Al Baqlani Ministry of Health Department of Public Health Laboratories Oman	*"Sincerely I had a wonderful experience with your institution both academically and socially"*
Dr. R. Nagaraj CCMB Hyderabad	*"The facilities and the work done in TRC is really impressive"*
Prof. K. Sankaran CBT - Anna University Chennai	*"A very exciting and stimulating experience going through the facilities and listening to the Director"*
Dr. Paul Kutyabami Makerere University Uganda.	*"I was impressed with what goes on at TRC. It will be good if what you do gets published for the world to benefit"*
Dr. P.M. Jester University of Alabama at Birmingham, USA	*"Your accomplishments are fantastic. I am honoured to have shared time with you"*
Dr.G.S.Chhatwal Director Microbiology HZI, Germany	*"A real nice centre for comprehensive research"*

Name	Remarks
Dr. Karyl Barron NIAID – NIH USA	*"I am most impressed with the facilities and look forward to seeing the results of the research"*
Dr. Hannan Mouse NIAD – NIH USA	*"Very Impressive"*

UK Parliamentarians visit

Tom Clarke House of Commons London Parliament	*"High standards of professionalism and commitment in action"*
Baroness Lindsay Northover Global Democrat Spokesperson International Development House of Lords London	*"It is very impressive indeed to see such wide range of outstanding research carried out here, and then able to influence what is happening in India and far wider"*
Ashok Kumar, MP House of Commons London	*"Highly committed individuals doing excellent work"*

Nick Herbert, MP House of Commons London Chariman, All-party Parliamentary Group on Global TB	*"Thank you for an extremely useful and informative visit"*
Ann Cryer, MP House of Commons London	*"Our morning at the TRC was extremely useful"*
Jeremy Hunt, MP House of Commons London	*"A fascinating insight into leading edge work to tackle TB and the relationship between HIV&TB. Keep up the good work"*
Sheila Davie & Louise Holly RESULTS, UK	*"Information that you have provided is invaluable for our advocacy work on TB"*

Nick Herbert MP
Arundel & South Downs

25 JUL 2007

HOUSE OF COMMONS
LONDON SW1A 0AA

Dr. P.R. Narayanan
Director
Tuberculosis Research Centre
Mayor V. R. Ramanathan Road
Chetput, Chennai 600 031
India

25 May 2007

Dear Dr Narayanan

I am so sorry not to have written sooner and on behalf of the members of the All-Party Parliamentary Group on Global TB who participated in the visit to India in March, thank you and your colleagues for giving up so much of your time to show us around the Tuberculosis Research Centre and for providing us with such comprehensive presentations.

It was very clear that in the 50 years since your establishment, the TRC has been a global leader in the field of research and its work has had a positive impact in addressing TB worldwide.

We would be extremely grateful if you would keep us informed of progress and developments in your work in the future and be in touch if there are any actions that the All-Party Parliamentary Group can take to support your efforts.

If you or any of your colleagues have the opportunity to travel to the UK in the future, please do let us know. We would be delighted to see you again.

May I wish you the very best of luck in your future activities.

With kind regards.

Yours sincerely

Nick Herbert MP

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RESULTS

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16 April 2007

Dear Dr Narayanan,

Re: Delegation of UK Parliamentarians to India

I am writing today to thank you very much for giving our delegation such an interesting presentation of the Tuberculosis Research Centre's activities.

Your presentation gave the delegates an excellent opportunity to better understand the scale of the tuberculosis epidemic in India. It also inspired the delegates to take action to support efforts to control the disease and I am pleased to tell you that they have promised to take a lead in the UK in advocating for solutions to the problem.

Delegations of parliamentarians are a very important part of our advocacy work and you greatly contributed to make this trip a very successful one.

Thank you again for your time and support. We very much look forward to working together again in the future.

With very kind regards,

Yours sincerely,

Sheila Davie
Executive Director

Louise Holly
Project Manager

ABBREVIATIONS

3TC	Lamivudine
A	Amikacin
ACR	Allelic Crystalline Protein
AFB	Acid Fast Bacilli
ANN	Artificial Neural Network
ART	Antiretroviral Therapy
ARTI	Acute Respiratory Tract Infection
ATP	Adenosine Triphosphate
ATT	Anti-TB treatment
AZT	Zidovudine
BCG	Bacillus Calmette Guerin
C	Capreomycine
CBA	Cytometric Bead Array
CFA	Culture Filtrate Antigen
CFP	Culture Filtrate Protein
CFU	Colony Forming Unit
CIC	Circulating Immune Comlex
CPC	Cetyl Pyridinium Chloride
CS	Chest Symptomatic
CTL	Cytotoxic T-lymphocytes
CXR	Chest X-ray
CYP2B6	Cytochrome P450 2B6
DC	Dendritic Cells
ddI	Didanosine
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
DOTS	Directly Observed Treatment Short-course
DRS	Drug Resistance Surveillance
DSP	Drug Susceptibility Profile
DST	Drug Susceptibility Testing
d4T	Stavudine
E	Ethambutol
EDTA	Ethylene Dianine Tetra-acetic Acid
EFV	Efavirenz
ELISA	Enzyme Linked Immunosorbent Assay
EQA	External Quality Assurance
ESAT	Early Secretory Antigen Target
Eth	Ethionamide
FDC	Fixed Dose Combinations
H	Isoniazid
HIV	Human Immuno Deficiency Virus
HIVVT	HIV Vaccine Trial
HHC	Healthy Household Contacts
HLA	Human Leucocyte Antigen
HPLC	High Performance Liquid Chromatography

IFN-γ	Interferon Gamma
IRL	Intermediate Reference Laboratory
K	Kanamycin
LJ	Lowenstein Jensen
LRP	Luciferase Reporter Phage
MAPK	Mitogen Activated Protein
MBL	Mannose Binding Lectin
MDR-TB	Multi-Drug Resistant Tuberculosis
MIC	Minimal Inhibitory Concentrate
MSM	Men having Sex with Men
MVA	Modified Vaccine Ankara
NHS	Normal Health Subjects
NNRTI	Non-NRTI
NVP	Nevirapine
O	Ofloxacin
ORF	Open Reading Frame
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain
PMA	Phorbol Myristate Acetate
PNB	Para Nitro Benzoic acid
PPD	Purified Protein Derivative
PPs	Private Practitioners
PST	Proportion Sensitivity Test
PTB	Pulmonary TB
QoL	Quality of Life
R	Rifampicin
RFLP	Reaction – Restriction Fragment Length Polymorphism
RLU	Relative Light Units
RNTCP	Revised National TB Control Programe
RT	Reverse Transcriptase
SLI	Standard of Living Index
Sm	Streptomycin
STDC	State TB Demonstration Centre
TB	Tuberculosis
TLR	Toll Like Receptors
TST	Tuberculin Skin Test
USAID	United States Agency for International Development
VDR	Vitamin-D Receptor
WHO	World Health Organization
Z	Pyrazinamide
ZN	Ziehl Neelsen

1. PHASE-I PREVENTIVE HIV VACCINE TRIAL

Ongoing studies:

A randomized, placebo-controlled, dosage-escalating phase I double-blinded study to evaluate the safety and immunogenicity of an MVA HIV-1 multigenic subtype C vaccine (TBC-M4) in HIV-uninfected, healthy volunteers

Background:

In view of the existing burden of HIV infection with its medical, social and economic dimensions, it was decided that efforts should be undertaken to test a vaccine against HIV as per the joint declaration by the NACO, ICMR and the International AIDS Vaccine Initiative. For this purpose, a phase I trial of a multigenic vaccine against HIV-1 subtype C using Modified Vaccinia Ankara (MVA) as the vector has been initiated.

Aims:

- To determine the safety and tolerability of three injections of the vaccine or placebo at two dose levels
- To determine the immunogenicity of this vaccine by measuring HIV-1 specific T- cell responses quantified by ELISPOT interferon-gamma (INF-γ) and serum binding antibody responses to HIV measured by ELISA

Methods:

A total of 32 volunteers were enrolled in two groups and followed up for a total of 18 months.

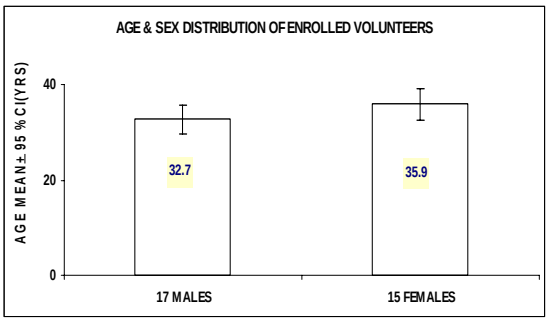
Results:

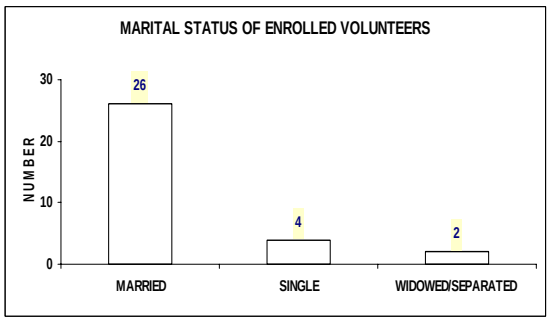
During the period under review, 32 volunteers were enrolled for the study comprising of 17 male and 15 female subjects. The demographic details of the enrolled volunteers are given in Fig.1.1-1.5. The intervention phase of the study consisting of the administration of 3 injections of the vaccine has been completed and the volunteers are in the follow up phase. A total of 457 of the scheduled

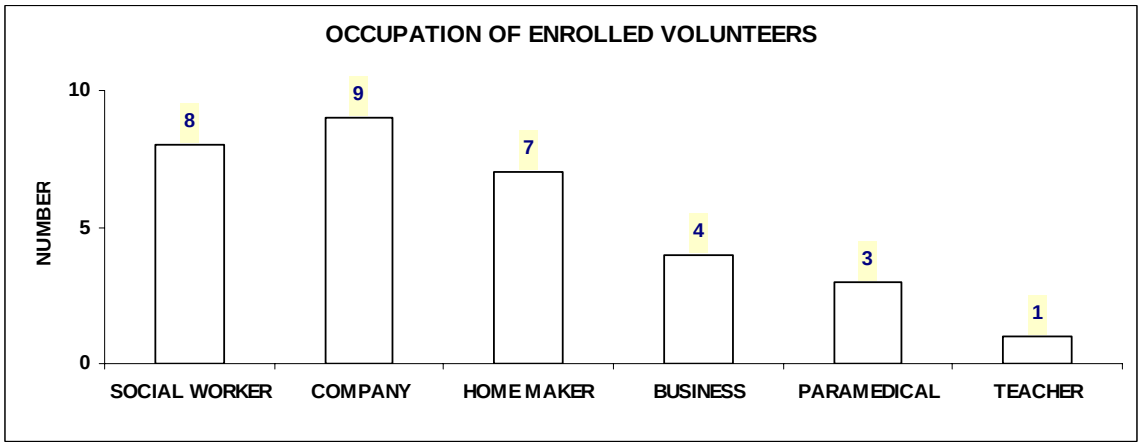
544 visits (84 %) have been completed. None of the volunteers has had any adverse or serious adverse effects related to the vaccine.

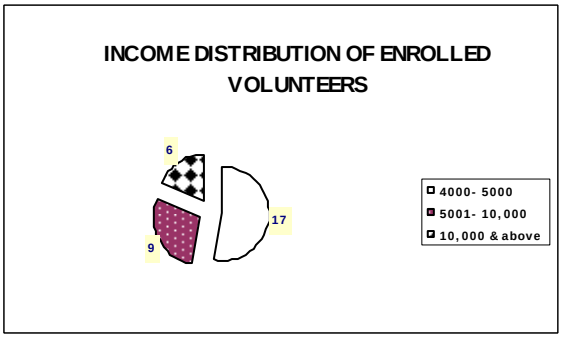
The volunteers are being followed up and the last visit is expected to be completed in February 2008.

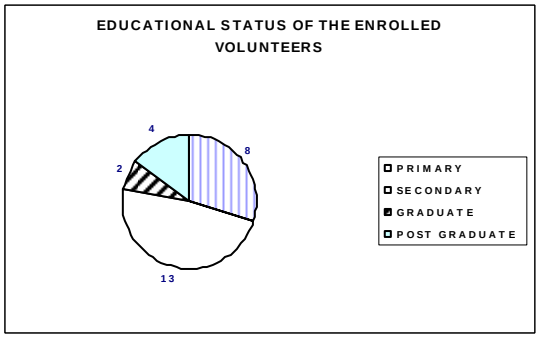
Fig. 1.1 – 1.5: Demographic Details of volunteers











Key message: The administration of 3 injections of MVA-TBC-M4 vaccine to healthy volunteers at two dose levels appears to be safe.

(PI: Dr. V.D. Ramanathan; ramanathanvd@trcchennai.in)

2. CLINICAL RESEARCH

Ongoing studies:

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

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 tuberculosis (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 immunomodulator to chemotherapy is well tolerated and does not increase the adverse reactions to the therapy.

Aim:

- To study the cure rate in Category II pulmonary TB patients after the addition of the *Mycobacterium w* vaccination to standard anti-TB drugs

Methods:

This study is planned as a double blinded, randomized, placebo controlled multicentric clinical trial. It is being initiated by the Department of Science and Technology. The patients are randomly chosen to receive either the vaccine or placebo along with the standard category II Revised National TB Control Programme (RNTCP) regimen. One hundred and twenty eight patients are proposed to be admitted to the trial.

Results:

The study was initiated in March 2006, and in one year period 57 patients were screened and 30 were included in the study (18 relapses, 4 failures and 8 treatment defaulters of Category I). Vaccine acceptability has been good among these patients. During the intensive treatment phase, 4 patients developed serious adverse reactions (2 rifampicin (R) - induced renal failure and 2 hepatotoxicity). This study is on going.

(PI: Dr.Rajeswari Ramachandran, rajeswarir@trcchennai.in: Funded by: Department of Biotechnology, Government of India.)

Evaluation of chemotherapy regimens for tuberculosis in HIV infected persons

Background:

The duration of anti-TB treatment (ATT) among 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 RNTCP in India and many countries.

Aims:

- To evaluate the efficacy of RNTCP treatment regimens among HIV patients infected with TB
- To compare the efficacy of a 6-month versus a 9-month intermittent anti-TB regimen among HIV positive patients with TB with respect to reduction in failure and relapses

Methods:

This study was planned as a prospective randomized controlled clinical trial to compare the efficacy of a 6 months' regimen (Regimen A-$2EHRZ_3/4RH_3$) with 9 months' regimen (Regimen B – $2EHRZ_3/7RH_3$). The dosages of ATT were as follows: ethambutol (E) 1200 mg, isoniazid (H) 600 mg, R 450 mg in patients with < 60 kg and 600 mg in patients with body weight $\geq$ 60 kg and pyrazinamide (Z) 1500 mg with pyridoxine 10 mg, given thrice weekly.

All HIV positive patients with pulmonary TB were diagnosed based on sputum smear and culture and lymphnodal TB by FNAC/Biopsy. Patients with exudative effusion suggestive of TB or miliary TB were included in the study. Randomization was done by permuted block scheme and stratified by CD4 cell count (< 200 & $\geq$ 200 cells/cu.mm), and smear grading (0, 1+ & 2+, 3+). Treatment was fully supervised for the first 2 months, then once a week. The intensive phase was extended by 4 weeks if sputum smears were positive at the end of 2^{nd} month. Patients were followed up every month with clinical examination, sputum AFB smear and culture for *M. tuberculosis*. Chest radiograph and CD4 counts were done at baseline, 2^{nd} 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 during follow-up for sputum culture positive cases and clinical improvement with radiological clearance for sputum culture negative cases. On -treatment analysis was performed.

Results:

Study population: Of the 334 receiving the study regimens, 65 were excluded in the efficacy analysis [2-early deaths, 14 cases who had confirmed non-tuberculous cause of death, 2-primary multidrug resistant-TB (MDR-TB), 17 cases -<80% treatment taken, 29 cases who had no definite evidence of TB and 1 had regimen changed due to need of ART containing nevirapine (NVP)]. Of the remaining 269 cases, 140 received regimen A and 129 received regimen B. Two hundred and one out of 269 cases had sputum culture confirmed pulmonary TB, of which 106 received regimen A and 95 received regimen B. Of the remaining 68 cases, 16 had TB lymphadenitis, 8 had TB pleural effusion, 4 had miliary TB (without culture confirmation) and 40 had smear and culture negative pulmonary TB with persistent X-ray findings and respiratory symptoms. The baseline characteristics of study population are given in table 2.1.

Table 1.1: Baseline Characteristics of study participants (n=273)

	Regimen A – 6M n = 140	Regimen B – 9M n = 129
Males (%)	81	74
Age (Years)	34 ± 7	34 ± 8
Weight (kg)	44 ± 7	44 ± 9
Sputum smear (% positive)	58%	57%
Sputum culture (% positive)	76%	74%
Median CD4	165 (range 82 – 308)	173 (range 90 – 295)

Outcome:

At the end of treatment, 126 (90%) in regimen A and 111 (86%) in regimen B had a favourable response. There was no statistically significant difference between the outcomes of two regimens. There were 8 and 11 bacteriological failures and 4 and 3 clinical deterioration in regimen A and regimen B respectively. One case in regimen A and 3 cases in regimen B died during treatment due to TB.

Regimen was changed due to toxicity to ATT in one patient in regimen A and 1 patient required NVP- containing ART along with change of ATT in regimen B.

The toxicity profile showed that 18% of patients had minor toxicity, 3 cases had ATT temporarily withheld due to jaundicewhich was successfully reintroduced. Only one had a permanent termination of the regimen due to cutaneous toxicity to ATT. Drug toxicity was similar in both the groups.

The results suggest that the 6-month and 9-month anti-TB regimens had similar efficacy, and addition of 3 months of ATT in the 9-month regimen as compared to 6 month regimen had no added benefit.

Follow Up: Follow up is ongoing to find out the relapse rate. Restriction fragment length polymorphism (RFLP) is being done to differentiate true relapses from re-infection.

(PI–Dr.Soumya Swaminathan; soumyas@trcchennai.in; Funded by ICMR Task Force on HIV-TB)

Preventive therapy for TB in HIV infected individuals

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 test (TST) (70% reduction in incidence, mortality reduced by 25%). Ideal duration of preventive therapy, especially in TB-endemic countries is not known.

Aims:

- To study the efficacy of two different preventive therapy regimens in HIV infected persons in reducing the incidence of TB and overall mortality
- To find out if a long duration/life long regimen with H daily is superior to a 6-month regimen of H and E

Outcome Measures:

1. Development of pulmonary or extra-pulmonary TB
2. Death due to TB

Study Design:

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

The treatment regimens were as follows:

1. E (800 mg) and H (300 mg) daily for six months, self-administered, collected once in fifteen days

2. H (300 mg) daily for 3 years (in lieu of life long prophylaxis) self administered, collected once in fifteen 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 fully investigated and treated appropriately. Any positive culture was subjected to drug susceptibility tests (DST). The cause was ascertained in all cases of death.

Results:

Of the 711 patients admitted to the study from March 2001- Sep 2005, 635 were eligible for analysis. The mean age, body weight, CD4 cell count and Mantoux were comparable in both the groups (Table 2.2). One hundred and seventy eight patients from the H arm and 175 from the EH arm have completed 36 months of follow-up as of March 2007. Thirty four patients in the H arm and 39 in the EH arm have been initiated on ART, because of falling CD4 cell counts. Eighteen patients in the EH arm and 18 in the H group developed active TB giving a breakdown rate of 1.76 / 100 person years respectively. Most of the breakdown in both the arms had occurred in the first 12 months. Number of deaths in the H arm were 21 (2.06 / 100 person years) as compared to 29 (2.84 / 100 person years) in the EH arm. Majority of the deaths had occurred between 12–24 months. The toxicity pattern was also similar in both the groups. In only one patient in the H arm, the treatment had to be terminated because of severe jaundice.

Table 2.2: Baseline Characteristics

	EH 6 MONTHS		H 36 MONTHS	
	n = 320		n = 315	
	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/cu.mm)	337±257	35-1125	330±224	12-1247
Mx (mm)	9 ± 10	0.0-40.0	8 ± 9	0.0-35.0

The interim findings suggest that the 6 months of EH regimen is as effective as 3 years of H in preventing TB among HIV-infected persons. Patients with lower CD4 cell counts are at higher risk of TB breakdown and death.

(PI: Dr.Soumya Swaminathan; soumyas@trcchennai.in. Funding: USAID)

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

Background:

People with HIV infection are at increased risk for developing TB. Though most patients respond well to ATT, they develop other opportunistic infections and deteriorate rapidly. Recurrence of TB is also more frequent. Timely use of ART co-administered with ATT could reduce these complications thereby improving mortality and long term outcome.

Aims:

Primary:

- To compare the efficacy and safety of two different once-daily ART regimen of didanosine + lamivudine (3TC) + efavirenz (EFV)/Nevirapine along with standard ATT in patients with HIV and TB with CD4< 250 cells/cu.mm.

Secondary:

- To compare the efficacy of ART given under partial supervision (observed three times a week) with unsupervised treatment (once a month supply)

Methods:

The study is a prospective randomized controlled clinical trial with patients given standard ATT ($2EHRZ_3/4HR_3$) as DOTS and randomized to receive ART regimen containing either NVP or EFV along with ddI and 3TC at the end of intensive phase of ATT. ART is given as DOTS for 6 months (8 months after start of ATT) and then patients are randomized if virologically suppressed to either DOTS or NON-DOTS arm. Recruitment of patients to the study is being done at Chennai, Madurai and Vellore. The primary outcome measure is suppression of viral load after 24 weeks of ART. A secondary outcome variable is to study the utility of DOT in this setting vs. self-administered ART at the end of 24 months. Pharmacokinetic studies are being planned concurrently to study the interaction between R and NVP/EFV.

Two hundred and seventy one patients have been screened (182 males and 89 females), out of which 81 have been recruited (63 males and 18 females) up to March 2007, 41 randomized to EFV regimen and 33 to NVP regimen. The mean weight and age of patients on admission was 42 (SD-8.6) and 35 (SD-7.4) respectively. The median CD4 cell counts of the study subjects was 84 cells (range: 3-232), the median viral load was 2,33, 000 copies/ml. The study is in progress.

(PI: Dr.Soumya Swaminathan; soumyas@trcchennai.in. Funding: National AIDS Control Organization, India)

3. SOCIOLOGICAL RESEARCH

Completed studies:

Understanding of gender differences in sex and sexuality among HIV positives – A study from Chennai, India

Background:

In the context of social development in the era of HIV/AIDS, a social and cultural understanding of gender particularly in the domain of sexuality is very important. This has a powerful influence on human behaviour.

Aim:

- To understand sexual behaviour patterns among HIV positive men and women

Methods:

This was a descriptive study on 203 seropositive men and women who attended the TRC out patient clinic and STD clinic in Government Hospital. A semi structured interview schedule was used to elicit data.

Results:

More men than women reported to having had premarital and extra marital relationships, most often with a commercial sex worker or a friend, and usually under the influence of alcohol. Fifty three percent of the women described the sexual relationship with their spouse as 'bad' compared to 23% of the men ($p<0.001$). Many women who refused sex said that their spouses reacted violently to their refusal. More than one-third of the women and 5% of the men were aware that their spouses were having extra marital relationship ($p<0.001$).

Key message: It is important to address sexual behaviour among HIV positive women and men. Focused interventions are needed to help men and women be aware of risky sexual behaviour and indulge in safe, healthy, satisfying sexual behaviour.

(PI: Dr. Beena E Thomas; beenathomas@trcchennai.in)

Ongoing studies:

Behavioral risk factors and HIV sero-prevalence among men who have sex with men (MSM) in Chennai

Background:

In India, the most typical route of transmission is through unprotected sexual intercourse. Men having sex with men (MSM) are a particularly high-risk group. It is estimated that many men engage in high-risk same-sex sexual behaviors. MSM are an "invisible" population in India and there is tremendous cultural pressure for MSM to get married and have children. This can place both the MSM themselves at risk as well as their wives and possibly their (unborn) children. They are therefore referred to as a bridge population. Despite the public health need, research on HIV prevalence and behavioral risk factors among MSM in India is negligible. The findings of this study will help to develop and test interventions for MSM.

Aim:

- To obtain correlates and frequency of HIV risk among MSM in Chennai and to estimate the prevalence of HIV in a sample of high risk MSM

Methods:

It is planned to recruit 200 MSM for the study. The study is in progress.

(PI: Dr. Beena E Thomas, beenathomas@trcchennai.in. Collaboration with Harvard University, USA)

Longitudinal study of the quality of life among patients on antiretroviral treatment

Background:

The quality of life (QOL) has emerged as a significant outcome in management of HIV/AIDS. QOL takes into account those dimensions directly affected by the disease and these include sociological, psychological, environmental and physical dimensions. Currently TRC is conducting a study to evaluate the safety and efficacy of two different ART regimens along with ATT in patients with HIV/TB co-infection.

Aim:

- To assess the QOL of all TB patients enrolled into the ART trial being conducted at TRC

Methods:

The QOL is being assessed at baseline, one year and two year time points. The WHO - BREF scale for QOL was used to assess the patients. So far, 35 patients have been enrolled and assessed for QOL. The study is ongoing.

(PI: Dr. Beena E Thomas, beenathomas@trcchennai.in)

4. EPIDEMIOLOGY & OPERATIONAL RESEARCH

Completed Studies:

Mortality surveys in Andhra Pradesh and Orissa:

Background:

The mortality data from these surveys will be used as baseline information for evaluating the impact of the RNTCP.

Aims:

- To estimate crude mortality rate in the states of Andhra Pradesh and Orissa
- To estimate the TB mortality rate among the general population aged ≥ 15 years

Sample Size, sampling design and methodology were described in detail in the previous annual report.

The coverages are presented in this report. The analysis is in progress.

Table 4.1: Sample units and coverage in Andhra Pradesh

State/ Districts	Total	Rural	Urban	Total	Alive	Dead	Moved out
Andhra Pradesh	**380**	**304**	**76**	**395886**	**389743**	**2344**	**3799**
Mehabub Nagar	69	62	07	74203	72711	448	1044
Khammam	51	41	10	52602	51916	276	410
Vizia Nagar	44	36	08	45728	45013	308	407
Krishna	82	56	26	85263	84186	472	605
Prakasam	60	51	09	61836	60587	353	896
Chittor	74	58	16	76254	75330	487	437

Table 4.2: Sample units and coverage in Orissa

State/ Districts	Total	Rural	Urban	Total	Alive	Dead	Moved out
Orissa	**380**	**310**	**70**	**390362**	**385160**	**2011**	**3191**
Bargarh	54	50	04	55169	54463	333	373
Debagarh	11	10	01	11263	11164	66	33
Sundargarh	73	48	25	74311	73337	437	537
Kendrapara	52	49	03	53594	52319	235	1040
Jagatsinghapur	42	38	04	43007	42447	164	396
Gajapati	21	19	02	21379	20912	152	315
Rayagada	33	28	05	33708	33316	252	140
Cuttack	94	68	26	97931	97202	372	357

(PI: Dr.C.Kolappan; kola155@trcchennai.in Funding: GFATM-CTD)

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 $\geq$ 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 X-ray abnormals 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:

Two disease surveys were already completed and the second resurvey was completed in June 2006. Coverage in the survey was above 90% for all investigations namely symptoms, X-ray and sputum examination as seen in Table 4.3. Overall, 346 persons were diagnosed to have smear/culture positive TB.

Table 4.3: 2nd Resurvey status

Activities	2nd resurvey
Eligible for symptom and X-ray	107676
Symptom screening	98498 (91%)
X-rayed	97229 (90%)
Sputum Eligible	12652
Sputum collected	11976 (95%)

The data analysis has been taken to estimate the decline in prevalence based on the findings of three surveys.

The 3rd resurvey was started in June 2006. This survey will confirm the decline in TB based on previous three surveys. The coverage in the present survey was above 90% for all investigations as shown in Table 4.4.

Table 4.4: 3rd Resurvey status (till March 2007)

Activities	3rd resurvey
Eligible for symptom and X-ray	32404
Symptom screening	30000 (93%)
X-rayed	29591 (91%)
Sputum eligible	4065
Sputum collected	3956 (97%)

Eighty seven were identified as cases either by smear, culture or both.

(PI: P.G.Gopi; gopipg@trcchennai.in)

Annual risk of tuberculosis infection in Chennai city

Background:

The tuberculin surveys and the computed Annual Risk of Tuberculosis Infection (ARTI) provide the indirect method of assessing the extent of TB infection in the community. The proportion infected was found to be higher in urban compared to rural children in a national sample survey on ARTI conducted during 2000-2003. In the south zone, the proportion infected was 8.8% (ARTI: 1.6%) in urban and 4.7% (ARTI: 0.8%) in rural. The urban population selected for the south zone included children from peri-urban area of Chennai city. Moreover, epidemiological information on TB in the city is very limited.

Aim:

- To conduct a tuberculin survey in Chennai city to provide a precise estimate of prevalence of infection and ARTI

Methods:

The sample size was estimated to be 7000 children aged between 1 to 9 years. A stratified cluster sampling methodology was adopted to select the sample. The number of slum and non-slum streets from each zone was selected based in the ratio of slum to non-slum population in the city (1:3). All children were tested with 1TU of PPD RT23 tuberculin vials and the reaction sizes were read after 72 hours.

Results:

Of the 7354 children tested, reaction was read in 7098 (96.5%) children. The mode at the right hand side is fairly located at 17mm. Using the mode at 17mm, the prevalence of infection among unvaccinated, vaccinated children and children irrespective of BCG scar was estimated to be 10.5, 9.2 and 9.5% respectively. The difference in the proportions of children infected among unvaccinated and vaccinated was not statistically significant. The computed ARTI was 2.0, 1.7 and 1.8% respectively. The infection among children in slum (11.1%) was significantly higher than that among children in non-slum (8.9%) ($p<0.01$).

Conclusion:

The risk of infection was significantly higher among children in slum area compared to non-slum area. This information can be used as baseline information for monitoring the epidemiological trends in future.

(PI: P.G.Gopi; gopipg@trcchennai.in)

Prevalence of tuberculosis in different economic strata: a community survey from south India

Background:

Series of community surveys on prevalence of pulmonary TB were undertaken in a rural community in south India. A prospective socio-economic survey was added to one of the surveys.

Aim:

- To measure the prevalence of TB in different economic strata

Methods:

Head of the house-holds included in the TB prevalence survey were interviewed to collect socio-economic data and assets owned by the family using a semi–structured questionnaire. Based on the data collected, standard of living index (SLI) was constructed. Cases of TB were identified by sputum smear examination among those with chest symptoms and/or abnormal shadows on X-rays. SLI was correlated with prevalence of TB.

Results:

The survey covered 32,780 households. The SLI was low, medium and high in 22%, 36% and 42% of the study population, respectively. The corresponding prevalence of TB was 343, 169 and 92 per 100,000, a statistically significant trend ($p<0.001$). Among the TB patients identified, 57% were from low SLI; prevalence of TB was higher amongst the landless, those who were living below poverty line and living in *katcha* houses.

Conclusion:

More than half of the TB patients in the prevalence survey were from low SLI, confirming that TB disproportionately affects the poor.

(PI – Dr. Rajeswari Ramachandran; rajeswarir@trcchennai.in. Funding WHO-USAID)

Ongoing studies:

Increased yield of smear positive pulmonary TB patients by screening patients with ≥ 2 weeks cough, compared to ≥ 3 weeks and adequacy of 2 sputum for diagnosis of sputum positive patients

Background:

RNTCP recommends diagnosis of pulmonary TB by examining three sputum smears for AFB from chest symptomatics (CSs) with cough of ≥ 3 weeks. A multi-centric study was undertaken by TRC to compare the yield of smear positive cases among CSs with cough of ≥ 2 weeks and ≥ 3 weeks. There was a 46% increase in the yield of sputum positive cases if duration of cough was reduced to 2 weeks. Various other studies have shown that ≥ 95% of pulmonary TB cases can be diagnosed by doing two smears examination. To validate the above findings the study was repeated in different settings in five geographical areas in the country.

Aims:

- To assess the yield of sputum positive cases among CSs with cough of ≥2 weeks compared to cough of ≥ 3 weeks
- To compare the efficacy of two smear examination instead of three smears, among CSs

Methods:

This was a cross sectional multicentric study carried out in five states conveniently selected in the country namely; Andhra Pradesh, Maharashtra, Orissa, West Bengal and Rajasthan where there was 100% coverage for RNTCP. From each of these states, three districts each were selected; with low, medium and high target achievements for case detection rates as per the RNTCP performance report, India, fourth quarter, 2004. A convenient sample of 90 primary and secondary level health facilities with high out-patient attendance were selected from these districts in order to obtain about 10,000 samples from each state.

All the health workers and medical officers were briefed about the purpose and trained in the procedures of the study. Supervisory visits were made to ensure good quality sputum smear microscopy and good coverage of all eligible patients. The patients were asked for their complaints first and for those who did

not give history of cough on his own, it was elicited by a direct question using a simple structured questionnaire. Three sputum specimens were collected from the eligible patients and smear microscopy was done as per the programme recommendation.

During the study period, 96,787 out patients were registered in the selected centers of 5 states. Among them 69,209 (72%) were new adult (aged ≥15 years) out patients. The study has been completed and data analysis is in progress.

(PI – Dr. Aleyamma Thomas; aleyammat@trcchennai.in; Funding: WHO-USAID)

Reasons for hospitalization of TB patients

Background:

DOTS strategy with decentralization of diagnosis and treatment of TB has been implemented under RNTCP in India since 1998. Uninterrupted supply of good quality drugs and quality assured sputum smear microscopy has been made available at the peripheral health institutes. Treatment is provided at a place convenient to the patient, by a provider, acceptable to the patient and accountable to health system. Still many TB patients are treated as in-patients in a TB hospital. The reasons for hospitalization of these patients, even after decentralization of management of TB have not been documented.

Aim:

- To find out the various reasons for hospitalization of TB patients

Methods:

Four hundred and fifty patients admitted in three TB hospitals of Tamil Nadu. (Government Hospital of Thoracic Medicine, Tambaram, Government, Tiruvoteeswarar Hospital, Otteri, Chennai, Government Thoracic Hospital, Austinpatti Thoppur, Madurai -150 patients from each hospital) were interviewed. The study period was from February 2006 to November 2006. The information was collected by using a semi-structured interview schedule. The clinical characteristics and medical details at the time of admission were collected from patients' case records by a Medical Officer. The socio economic characteristics were collected by a Medical Social Worker by using a questionnaire.

A total of 489 patients admitted in 3 hospitals were assessed. Of these, 95 were excluded from the analysis as they did not have active TB requiring treatment.

Hence 394 patients who were on treatment for TB are included for the analysis. The intake to the study is over. The data analysis is in progress.

(PI – Dr. Pauline Joseph; josephp@trcchennai.in; Funding: WHO-USAID)

Drug susceptibility profile of *M. tuberculosis* isolates from patients who remain smear positive at fourth month or later during treatment with category-II regimen

Background:

All new TB patients are treated with either Category-I or Category-III regimen in the RNTCP in India. Category II ($2EHRZS_3$/ $1EHRZ_3$/ $5HRE_3$) regimen is the re-treatment regimen of RNTCP. Patients who fail to Category II regimen have to be referred to a speciality centre for culture and DST and for further management. The drug susceptibility profile (DSP) of bacilli from these patients has not been documented by any study. Information about this will be of help for evolving standardized regimens for these patients under programme conditions.

Aim:

- To assess the drug susceptibility profile of *M. tuberculosis* isolates from patients who remain smear positive at fourth month or later during treatment with Category II regimen

Methods:

Patients for this study are being recruited from Tiruvallur District (all 6 TB units) and Chennai Corporation area. Two sputum specimens are being collected for drug sensitive test from patients on Category-II regimen, who remain smear positive at fourth month or later. It is planned to include 250 patients to the study. The study started in May 2006. So far, 120 patients have been enrolled to the study. The study is in progress.

(PI – Dr. Pauline Joseph; josephp@trcchennai.in; Funding: WHO-USAID)

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 developed active disease with in 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 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 the information on the etiological profile of respiratory infections and their sensitivity pattern and appropriateness of antibiotic algorithm

Methods:

Patients with cough of 3 weeks or more and 3 smears negative by sputum microscopy are being recruited to the study. It is proposed to admit 700 patients to each antibiotic arm.

Antibiotic regimen

Patients are being randomly allocated to one of the following antibiotic regimens:

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

Recruitment of patients to the study is in progress.

(PI – Dr. D. Baskaran; baskar.d@trcchennai.in.)

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

Background:

Since the advent of HIV there has been a disproportionate increase in the reported rates of smear negative TB. There is a delay in diagnosing this condition leading to higher mortality rates. The WHO has recommended certain

revisions in the existing guidelines to the diagnosis of smear negative TB in HIV patients. This differs from the RNTCP guidelines that are currently being practiced in India. We have tried to amalgamate the two (WHO & RNTCP guidelines) and develop a new algorithm to diagnose smear negative TB in HIV positive patients.

Aims:

- To develop & evaluate a diagnostic algorithm for HIV positive persons suspected to have TB, but who are smear negative for AFB during the initial screening for TB
- To determine the utility of initial chest x-ray (CXR) & sputum culture, in the RNTCP diagnostic algorithm in identifying TB cases among the initial smear negative HIV positive chest symptomatic

Patient enrollment to this study started in February 2007 and 46 patients have been enrolled in to the study up to March 2007.

(PI: Dr.Padmapriyadarsini, padmapriyadarsinic@trcchennai.in.; Funding: WHO-USAID Collaboration with NARI, Pune)

Survey of the prevalence of anti-tuberculosis drug resistance in Gujarat state

Background:

Drug resistance surveillance (DRS) in the state of Gujarat is being conducted to obtain baseline information on the level of drug resistance for four primary anti-TB drugs after implementation of RNTCP in the state.

Aims:

- To determine the prevalence of drug resistance in 'new' smear positive pulmonary TB patients
- To determine the prevalence of drug resistance in 'previously treated' smear positive pulmonary TB patients
- To establish the foundation for routine surveillance of drug resistance in order to observe trends in drug resistance

Methods:

The study was a population based study and conducted during the period August 2005 - May 2006. This period included patient intake, data analysis, submission of technical and financial reports, report on the performance of culture laboratory

at State TB Training and Demonstration Centre (STDC) (Ahmedabad), and the External Quality Assurance (EQA) of smear microscopy, culture and DST of STDC and Intermediate Reference Laboratory (IRL).

Culture and Susceptibility testing was done as per the National DRS protocol developed by the Central TB division. Briefly, the specimens received in 1% cetyl pyridinium chloride (CPC) - 2% sodium chloride (NaCl) was centrifuged, washed and inoculated on to Lowenstein Jensen (LJ) media and incubated at 37^{o}C for eight weeks. Reading of growth was recorded every week. Identification was done by growth on 500 μg/ml of para-nitro benzoic acid (PNB) and niacin test. Economic variant of Indirect Proportion method was followed for susceptibility testing for four primary drugs H, R, Streptomycin (Sm) and E as per the DRS protocol.

Results:

The prevalence of MDR among new cases was 2.4% and 17% among previously treated cases. The resistance pattern among new and re-treated cases is given in table 4.5.

Table 4.5: Resistance pattern among new and re-treated cases

	New Case	Re-treated Case
Total patients with DST results	1571	1045
Total Susceptible	1236	562
Total Any resistance	335	483
HR resistance	37	180

The intake into the study is completed and data analysis is in progress.

(PI: Dr.Ranjani Ramachandran; ranjanir@trcchennai.in; Funding: CTD)

5. APPLIED RESEARCH

Completed Studies:

Pot-staining of sputum for detection of acid-fast bacilli

Background:

For over a century the diagnosis of pulmonary TB is being confirmed by detection of acid fast bacilli (AFB) in direct smears of sputum made on glass slides. Good laboratory practices have to be followed while making direct smears from the mucopurulent portion of sputum on glass slides to avoid laboratory acquired TB infection. Generally, making direct smears from sputum is considered hazardous by laboratory technicians working in developing countries with limited facilities. In some higher level laboratories, smears are made from deposits after processing sputum samples. These deposit smears, in addition to generating aerosols often get peeled off from the slides resulting in false-negative results. In Ziehl-Neelsen (ZN) method, the carbol-fuchsin solution poured on the slide is to be heated until vapour rises and should be allowed to remain that way for five minutes. In order to overcome these problems, there is a felt need for a new technique to stain the AFB, which is both simple and non-hazardous. Recently, it has been shown that the deposit of sputum sample, obtained after decontamination with 4% sodium hydroxide and concentrated by centrifugation, can be stained with 1% carbol-fuchsin in its container and its smears made subsequently on glass slide can be decolorised and counter-stained by the procedures followed in ZN method.

Aims:

- To stain the sputum samples in their containers and subsequently decolorise and counter-stain their smears (pot-smears) for detection of AFB

- To compare results of pot-smears with their corresponding direct-smears stained by the hot ZN method

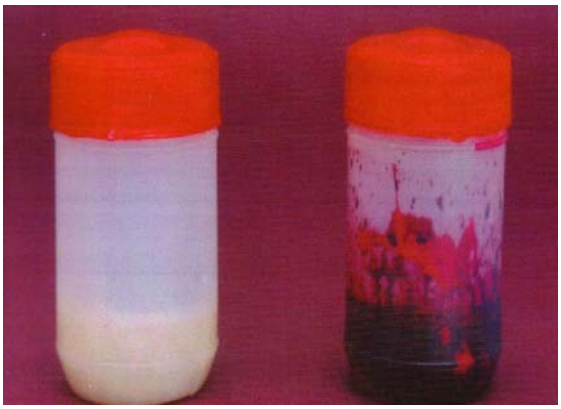
Methods:

Seven hundred and twenty five direct smears made from sputum samples of pulmonary TB patients were stained by the hot ZN method for detection of AFB. Each of the above sputum sample was then mixed well in its container with 'phenol ammonium sulphate basic-fuchsin' solution (Fig.5.1) and its pot-smear, made after 1 hour (Fig.5.2), was then decolorised and counter-stained by the same procedures followed in ZN method (Fig.5.3). The coded direct and the corresponding pot smears were graded and the results compared.

Results:

Of the 725 samples, 212 and 213 were AFB positive by direct and flocculate-smears respectively and the difference observed in the smear results was not statistically significant.

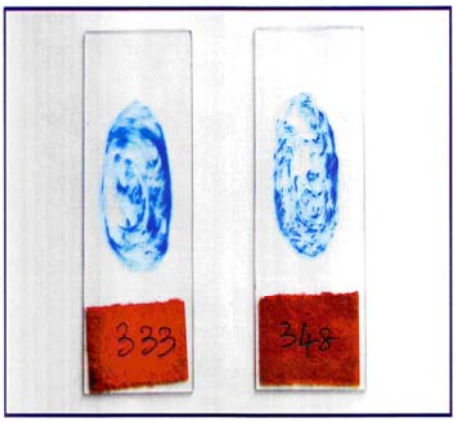
Fig.5.1 : Pot-stained sputum samples



Sputum sample | Sputum sample with the staining solution

Fig.5.2: Smears made from pot-stained sputum samples

Fig.5.3: Counter stained pot-smears



Conclusion:

The sputum samples in their containers can be stained with 'phenol ammonium sulphate basic-fuchsin' solution and their smears made subsequently on glass slides can then be decolorised and counter-stained for detection of AFB. The results of pot-smears and direct smears are comparable.

(PI: Dr.N.Selvakumar; selvakumarn@trcchennai.org.)

Standardisation and evaluation of optimized diagnostic Luciferase reporter phage (LRP) Assay

Background:

Luciferase reporter phage assay (LRP) has the potential for use in diagnosis and drug susceptibility detection of *M. tuberculosis*. Though reported to have high specificity, LRP assay lacks sensitivity. An attempt was made to optimize the assay format to improve its sensitivity. One approach was to delay the premature lysis of the infected host cell through lysis inhibition. Subsequent infection of the host cells prior to cell lysis, results in continuous availability of cellular adenosine triphosphate (ATP) and increased copy number of the enzyme. A second approach was to increase the permeability of the cell membrane to facilitate transport of the substrate D-luciferin using dimethyl sulfoxide (DMSO).

Aim:

- To optimize the LRP assay format to improve its sensitivity

Methods:

Standardization experiments were set up with culture suspensions of *M. tuberculosis* H_{37}Rv, multi-drug resistant and Sm, H, R, E (SHRE) sensitive clinical isolates. Cells infected by lytic LRP construct (phAE129) and lysogenic construct (phAETRC16) were reinfected with phAE129 at different time points and the cell phage mixtures were incubated at 37^{o}C. Control was setup with mycobacteriophage (MP) buffer instead of phAE129 at the time of reinfection. Photons released were measured at different time intervals as relative light units (RLU) in the luminometer.

D-Luciferin reconstituted in DMSO and made up in sodium citrate buffer was compared with that reconstituted in distilled water and made up in sodium citrate buffer. The cell phage suspension was prepared as per standard protocol for LRP assay using *M. smegmatis* mc^{2}155 and phAE129.

Using the above modifications in the assay format, LRP assay was done in sputum deposits of 67 samples processed by modified Petroff's method. About 300 µl of deposit was added to 3 ml of modified 12B medium supplemented with antibiotics PANTA and incubated at 37^{o}C. A 1/10 dilution of the deposit and LJ slopes were inoculated. On days 3, 6, 9 and 12, LRP assay was set up in the optimized format and readings were taken at different time points. LJ slopes were read every week till eight weeks.

Results:

Lysis inhibition experiments revealed that second infection of the cells by phage produced marked increase in light output when compared to the control. D-Luciferin in 2.5% DMSO gave one log higher RLU than D-luciferin by standard preparation.

Among the 67 samples tested, conventional LJ culture was positive for 25 samples and negative for 42 samples. LRP assay was positive for 28 and negative for 39 samples. Twenty samples were positive and 34 were negative by both methods. The overall sensitivity of LRP assay was 80% (20/25) and specificity 81% (34/42) (Table 5.1). Time to detection ranged between one to four

weeks with a median at 2.3 weeks by LJ and that of LRP ranged between 1 to 12 days with the median at 5.7 days.

Table 5.1: Comparison of optimized LRP with conventional LJ method

		LJ		
		POS	NEG	TOTAL
LRP	POS	20	8	28
	NEG	5	34	39
	TOTAL	25	42	67

Conclusion:

Optimized LRP assay shows promise as a rapid assay for diagnosis of TB from sputum samples. Further evaluation with more number of samples can be done to include it as a standard diagnostic test.

(PI: Dr.Vanaja Kumar; vanajakumar@trcchennai.in)

Application of LRP assay for screening compounds from natural sources for antimycobacterial activity

Background:

The resurgence of TB is one of the most serious public health challenges of the 21st century. The need for discovering novel antimycobacterial compounds has been strongly felt due to the emergence of strains resistant to classic anti-TB drugs. Natural and semisynthetic compounds from actinomycetes and plants have been shown to have high levels of antimycobacterial activity. LRP assay has been employed for rapid screening of such compounds for antimycobacterial activity because of its simplicity and economy.

Aim:

- To study the effectiveness of nine extracts from extreme ecosystem actinomycetes and 25 plant-derived chalcone semisynthetic compounds against strains of *M.tuberculosis*

Methods:

Laboratory and clinical strains of *M. tuberculosis* were grown in compound-containing and compound-free Middlebrook 7H9 complete medium for three days at 37^{o}C. Concentrations of 50 and 100 µg/ml were tested for chalcone derivatives while actinomycetes extracts were tested in 100 and 500 µg/ml concentrations. Rifampicin was included as assay control. LRP phAE129 was added and the samples were incubated for three hours. Equal volume of the cell phage mixture was mixed with 0.3 mM D-Luciferin in 0.05M sodium citrate buffer of pH 4.5. Light output was immediately measured as RLU in the luminometer at 10 seconds integration. Compounds exhibiting a reduction of 50% or more in RLU in the test vials compared to that of the control were considered to have antimycobacterial activity.

Results:

Among nine extracts of actinomycetes, four were found to have antimycobacterial activity at a concentration of 100 µg/ml while six of them inhibited growth at a concentration of 500µg/ml (Table 5.2). Out of the 25 Chalcones derivatives, 10 and 17 compounds showed antimycobacterial activity at a concentration of 100 µg/ml and 500 µg/ml respectively (Table 5.3).

Table 5.2: Antimycobacterial activity of Actinomycetes

Extract	% reduction in RLU	
	100 µg/ml	500 µg/ml
D10- A	84.65	87.65
D10- B	84.17	85.97
D10 –C	79.02	81.77
D5	0	88.13
K29	0	0
KA1	26.98	85.25
ANS2	64.15	78.18
AD10	0	0
K24 A	0	0
R (2 µg/ml)	84.41	

Table 5.3: Antimycobaterial activity of chalcone derivatives

Compounds	% reduction in RLU	
	50 µg/ml	100 µg/ml
C1	70.02	70.18
C2	46.29	66.51
C3	30.95	48.06
C4	41.93	50.64
C5	31.59	98.86
C6	7.36	17.38
C7	68.41	90.52
C8	16.88	51.59
C9	24.06	48.83
C10	0	0
C11	58.75	65.08
C12	0	0
C13	66.07	72.98
C14	0	21.87
C15	43.16	63.9
C16	29.25	55.16
C17	0	12.17
C18	92.19	96.45
C19	64.61	89.27
C20	90.06	96.22
C21	94.3	98.15
C22	31.55	33.8
C23	60.24	98.52
C24	98.9	99.04
C25	84.16	97.94
R (2 µg/ml)	84.41	

Conclusion:

Actinomycetes and chalcone derivatives have moderate to high inhibitory activity against *M. tuberculosis*. They may be ideal candidates for development of anti-TB agents.

(PI: Dr. Vanaja Kumar; vanajakumar@trcchennai.in)

Use of phagebiotics for growing tubercle bacilli in liquid medium from sputum processed by Chitin and Petroff's methods

Background:

Growing *M. tuberculosis* in liquid medium is mandatory for rapid diagnosis of TB. Overgrowth of normal flora escaping the action of sputum processing chemical is the major problem in liquid media used for the primary isolation of tubercle bacilli, which affects the sensitivity of any rapid assay. Phage cocktail (Phagebiotics) substituting the use of antibiotics to control the overgrowth of normal flora in sputum samples has been established and it forms a novel, bio-friendly approach to tackle the problem of non-mycobacterial contaminants. It is essential to know the effect of phagebiotics on the growth and retrieval of *M. tuberculosis* to use it in rapid diagnostic assays.

Aim:

- To evaluate the effect of phagebiotics on sputum samples processed by Chitin and modified Petroff's methods for the early and better recovery of tubercle bacilli on LJ medium

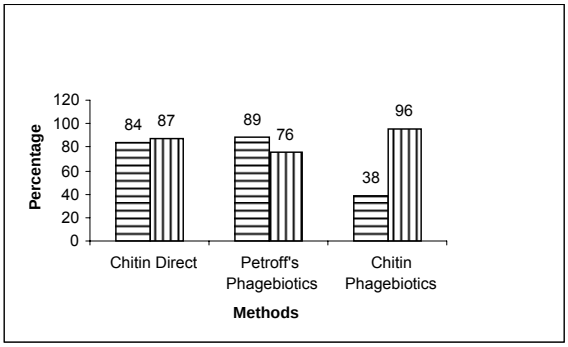
Methods:

A total of 120 sputum samples were collected from Otteri TB hospital, Chennai and divided into two parts and randomized. One part was processed by modified Petroff's method and other by Chitin method. One loopful each of the sputum deposits were inoculated onto two LJ medium slopes, two ml of phagebiotics was added to rest of the sputum deposits and incubated at 37^{o}C for 18-24 hours. Later, one loopful from each of the deposits was inoculated onto two LJ slopes, randomized, incubated at 37^{o}C and read every week up to eight weeks.

Results:

Culturing *M. tuberculosis* after processing by modified Petroff's method is considered as gold standard. Culture results of chitin direct, chitin-phagebiotics and Petroff's-phagebiotics were compared with Petroff's culture. Sensitivity and specificity of Petroff's-phagebiotics was 89% and 76% respectively while that of chitin direct method was 84% and 87% respectively (Fig 5.4). Only 38% of the real positives were picked up by chitin-phagebiotics while the specificity was as high as 96%. Chitin being acidic, sputum deposits processed with chitin had a pH of ~ 5.5 after the addition of phagebiotics while those processed with 4% sodium hydroxide were at ~ 8.5. Overnight incubation of sputum deposits in acidic pH probably lead to the killing of tubercle bacilli.

Fig. 5.4: Sensitivity and specifity of culture methods in comparison with Petroff's method



Conclusion:

Sputum processing with chitin resulted in moderately better retrieval of tubercle bacilli, while overnight incubation with phagebiotics reduced the sensitivity much further. Reduction in pH and dilution of reaction mixture by the addition of phagebiotics lead to the reduction in number of viable bacilli. Further studies are planned with neutralized deposits.

(PI: Dr. Vanajakumar; vanajakumar@trcchennai.in)

Evaluation of nitrate reductase assay as rapid inexpensive method for identification of multi drug resistant tuberculosis

Background:

Multi drug resistant TB is an increasing public health concern globally, especially in low-income countries. Current DST methods for *M. tuberculosis* are either expensive and automated or time consuming and conventional. There is a great need for a rapid, reliable and inexpensive method for DST.

Aim:

- To compare the nitrate reductase assay (NRA), a colorimetric method based on the ability of *M. tuberculosis* to reduce nitrate to nitrite

Methods:

A panel of 100 *M. tuberculosis* strains with various resistance patterns was tested using the NRA. Minimal inhibitory concentration (MIC), proportion sensitivity test (PST) and BACTEC 460 methods were used for comparison. Results of NRA were read after incubation for 7 to 14 days.

Results:

The susceptibility results of 100 *M. tuberculosis* strains against Sm, H, R, E and ofloxacin (O) gave an overall agreement of 91% between the NRA and BACTEC - 460, 95% for PST and 92% for MIC. The sensitivity of NRA compared with BACTEC - 460, PST and MIC was 98%, 98%, 100% for H, 97%, 98%, 98% for R. The specificity was 98%, 96% and 96% for H, 95%, 98% and 98% for R. For majority of cases, the results were available in 10 days.

Conclusion:

This study showed that the NRA method is rapid and inexpensive and could correctly identify most resistant and sensitive *M. tuberculosis* strains. The agreement between NRA and other methods suggests that this test has the potential to become an alternative for rapid and accurate detection of MDR-TB, particularly in resource poor settings.

(PI: Dr. Ranjani Ramachandran; ranjanir@trcchennai.in)

Characterization of HIV-1 isolates from antiretroviral naïve children in south India

Background:

Although HIV/AIDS can affect people of all ages, this epidemic has shown a rapid shift towards women and young people, with about half of the new infections occurring in ages below 25. Knowledge about the prevalence and pattern of HIV drug resistance in the community is important for formulating ART strategies for countries/populations. Most studies of antiretroviral drug resistance have been in adults; however, these results may not be directly applicable to pediatric cohorts, since factors that influence selection of drug resistant variants may be different in children. The factors responsible for such variations include the heterogeneity of HIV-1, the pharmacokinetics of antiretroviral drugs across different age groups, and unique barriers to adherence to treatment regimens.

Aims:

- To characterize the distribution of HIV-1 subtypes among treatment-naïve children in Chennai, south India
- To describe polymorphisms in reverse transcriptase (RT) and protease genes
- To detect the presence of drug resistant mutations if any

Methods:

Pattern of polymorphism and potential drug resistance mutations were evaluated in HIV-1 isolates from 48 children naïve to ART, attending the outpatient clinics of the TRC. The samples were subjected to genotyping of RT and protease genes.

Results and conclusion:

All the isolates were identified to be HIV-1 subtype C. All samples showed significant polymorphisms in both RT and protease genes, but none had major drug resistance mutation. Hence, the currently recommended generic first line antiretroviral drug combination is an appropriate treatment strategy for ART naïve HIV-1 infected children in India.

(PI: Dr.Soumya Swaminathan; soumyas@trcchennai.in.)

Study of *CYP2B6* polymorphism (G516T) and *ABCB1* polymorphism (C3435T) in HIV-1-infected individuals from south India

Background:

The NNRTIs, EFV and NVP are used as a first-line treatment of HIV-infected patients. Plasma concentrations of EFV and NVP are known for a high degree of inter-patient variability. These variations could be due to single nucleotide polymorphisms in genes coding for certain drug-metabolising enzymes or transporter proteins. A G to T change at position 516 (that is, 516 G>T) of *CYP2B6* gene and a C to T change at position 3435 of *ABCB1* gene could influence plasma concentrations of EFV and NVP. We studied the influence of *CYP2B6* G516T and *ABCB1* C3435T gene polymorphisms on EFV and NVP plasma concentrations in HIV-infected patients in south India, and also the prevalence of this polymorphism in the same population.

Methods:

The study participants comprised of HIV-infected individuals and apparently healthy subjects. The HIV-infected persons were receiving ART with EFV or NVP-based HAART regimens from the Government Hospital of Thoracic Medicine, Tambaram, Chennai. The patients were receiving EFV (600 mg once daily) or NVP (200 mg bi-daily) along with 3TC (150 mg bi-daily) and stavudine (d4T) (30/40 mg bi-daily)/zidovudine (AZT) (300 mg bi-daily). None of the patients was receiving any co-medications that would alter the blood levels of EFV or NVP. The healthy subjects consisted of staff working at the centre.

Venous blood (2 ml each) was collected in both heparin - and ethylene diamine tetra acetic acid (EDTA) - containing vacutainers from all the patients, while in the case of healthy subjects, blood was collected only in EDTA vacutainers. The time of blood collection was chosen in such a way that it represented 12-hour concentration for EFV and 2-hour concentration for NVP. The EDTA sample was used for DNA extraction and genotyping of *CYP2B6* and *ABCB1* polymorphism by PCR-RFLP analysis. The different genotypes of *CYP2B6* (GG, GT & TT) and *ABCB1* (CC, CT & TT) were identified based on the number and size of the fragments. Estimation of EFV and NVP in heparinised plasma was undertaken by high performance liquid chromatography (HPLC).

Results:

***CYP2B6* G516T polymorphism:** A total of 184 subjects took part in the study (130 HIV-1-infected persons and 54 healthy subjects). Of them, 50 were of the GG genotype (27%), 63 belonged to the GT genotype (34%) and 71 belonged to the TT genotype (39%). The G and T allele frequencies were 0.44 and 0.56 respectively.

Among the 130 HIV-infected persons, 64 were receiving treatment with EFV and 66 with NVP-containing regimens. The mean 12-hour plasma EFV concentration in the GG, GT and TT genotypes were 1.85, 2.0 and 6.44 µg/ml respectively (Table 5.4). The TT genotypes had a significantly higher plasma EFV concentration than the GG and GT genotypes ($p<0.01$). The mean 2-hour NVP blood levels for GG, GT and TT genotypes were 7.6, 7.95 and 10.53 µg/ml respectively (Table 5.4). The plasma NVP concentrations were significantly higher in the TT genotype compared to GG and GT genotypes ($p<0.05$).

***ABCB1* C3435T polymorphism:** This polymorphism was studied in a total of 179 subjects (126 HIV-1-infected persons and 53 healthy subjects). Of the 179 subjects, 78 were of the TT genotype (44%), 74 belonged to the CT genotype (41%) and 27 belonged to the CC genotype (15%). The C and T allele frequencies were 0.36 and 0.64 respectively.

Among the 126 HIV-infected persons, 55 were receiving treatment with EFV and 71 with NVP-containing regimens. The mean 12-hour plasma EFV concentration in the CC, CT and TT genotypes were 5.22, 3.5 and 2.48 µg/ml respectively (Fig. 5.5a). Although plasma EFV concentrations were highest in the CC genotype, followed by CT and TT genotypes, the differences failed to attain statistical significance. The mean 2-hour NVP blood levels for CC, CT and TT genotypes were 8.33, 8.99 and 7.5 µg/ml respectively (Fig. 5.5b). The plasma NVP concentrations were lower in the TT genotype compared to CC and CT genotypes. However, none of the differences was statistically significant.

Conclusions:

The study data show that frequency of *CYP2B6* G516T and *ABCB1* C3435T polymorphisms are high in the ethnic south Indian population. TT genotypes of the *CYP2B6* polymorphism have elevated plasma concentrations of EFV and NVP. With respect to *ABCB1* C3435T polymorphism, a trend in plasma EFV

concentrations was observed; patients with the CC genotype having the highest values followed by CT and TT genotypes. These findings suggest that inter-individual variations in plasma concentrations of EFV and NVP could be due to genetic variations in the *CYP2B6* gene and to a lesser extent *ABCB1* gene.

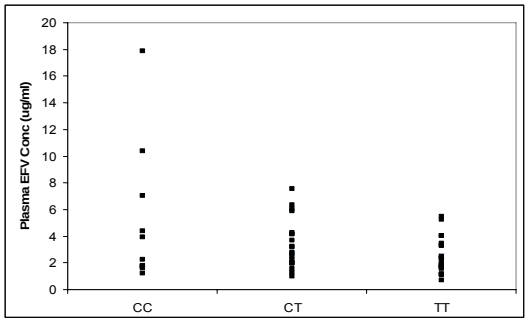
Table 5.4: Influence of *CYP2B6* polymorphism on plasma EFV & NVP

	Mean ± SD (µg/ml)	
Genotype	12-hr EFV (n=64)	2 hr-NVP (n=66)
GG	1.85 ± 1.15 (18)	7.60 ± 1.59 (19)
GT	2.00 ± 0.72 (19)	7.95 ± 2.82 (22)
TT	6.44 ± 2.63* (27)	10.53 ± 2.64* (25)

p <0.05 vs GG & GT
n given in parantheses

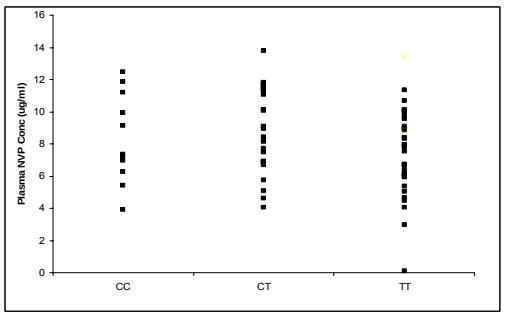
Fig. 5.5: Plasma concentrations of EFV (a) and NVP (b) in the different genotypes of *ABCB1* C3435T

a.



Genotypes

b.



Genotypes

(PI: Dr. Geetha Ramachandran; geethar@trcchennai.in)

Effect of anti-coagulants on plasma nevirapine and efavirenz concentrations

Background:

Plasma concentrations of the NNRTIs, NVP and EFV are reported to correlate with antiviral effect and possibly toxicity. Heparin is routinely used as an anticoagulant for plasma drug estimations, while EDTA is used for viral load and CD4 cell count measurements. Use of EDTA plasma for drug estimations, if found suitable, would have several practical advantages. Most of the developing countries do not have state-of-the-art sample collection and/or storage capabilities. Hence more data are needed on how different anticoagulants influence NVP and EFV concentrations.

Aim:

- To compare NVP and EFV concentrations in paired heparin and EDTA plasma in HIV-infected individuals undergoing ART

Methods:

The study was conducted in HIV-infected individuals who were receiving ART at the Government Hospital of Thoracic Medicine, Tambaram, Chennai. Their treatment consisted of NVP or EFV along with 3TC and d4T/AZT. Blood draws were made at the same time in heparin and EDTA vacutainers. Plasma concentrations of NVP and EFV were estimated according to validated methods by HPLC.

Results:

One hundred HIV-infected patients took part in the study. Of them, equal numbers were receiving NVP and EFV. The time of blood collection was at 2 and 12 hours respectively after NVP and EFV drug intake. The mean NVP concentrations in heparinised and EDTA plasma were 8.79 and 8.59 µg/ml respectively (Table 5.5). This difference was not statistically significant ($p=0.203$). In the case of EFV, the corresponding values were 3.03 and 2.78 µg/ml respectively (Table 5.5); the difference was statistically significant ($p<0.05$); However, the percent variation was less than 10%.

Table 5.5: Efavirenz and Nevirapine concentrations in Heparin and EDTA plasma

Variables	Nevirapine (n = 50)		Efavirenz (n = 50)	
	Heparin	EDTA	Heparin	EDTA
Mean	8.79	8.59	3.03	2.78
SD	3.16	3.02	3.08	2.79
Median	9.02	8.88	2.03	1.88
Range	0.10 – 14.82	0.12 – 15.36	0.11 – 16.22	0.11 – 12.65
P	0.203 (NS)		< 0.05 (S)	

Conclusion:

Plasma NVP levels did not differ when heparin or EDTA was used as anticoagulant. However, in the case of EFV, the difference between heparin and EDTA plasma was significant. Hence for plasma NVP determination, blood collected in heparin or EDTA vacutainers could be used.

(PI: Dr. A.K. Hemanth Kumar; hemanthkumarak@trcchennai.in)

Steady state pharmacokinetics of efavirenz in HIV-infected patients in south India

Background:

Concomitant administration of HAART and anti-TB medications is often difficult because of drug-drug interactions and the adverse effects profile. Efavirenz, a NNRTI has been recommended as a first line option in ART and the preferential choice in TB and HIV co-infected patients. The dosage of EFV when co-administered with R is a matter of debate.

Aim:

- To study the influence of sex, body weight, *CYP2B6* G516T polymorphism and R co-administration on the steady state pharmacokinetics of EFV in HIV-1-infected patients in south India

Methods:

Thirty four HIV-1-infected patients (25 males; 9 females) receiving ART with EFV (600mg once daily)-containing regimen at the Government Hospital of Thoracic Medicine, Tambaram, Chennai were recruited into the study. Of them, 15 had active TB and were receiving treatment with R-containing regimens (450/600 mg thrice weekly). The HIV-TB co-infected patients were investigated on two occasions, while receiving ART and ATT, and at least one month after stopping ATT. Serial blood collections predosing and at 1, 2, 4, 6, 8, 12 and 24 hours post dosing were collected in heparinised vacutainer tubes. An additional sample was collected in an EDTA vacutainer tube at any time point. Plasma EFV was estimated in the heparin samples by HPLC. The EDTA blood sample was used for DNA extraction and genotyping of *CYP2B6* G516T polymorphism by PCR-RFLP analysis.

Results:

Males and females did not significantly differ in peak concentration (C_{max}), trough concentration (C_{min}) and exposure (AUC_{0-24}) of EFV. Patients with body weight > 50 kg (n=14) had lower C_{max} (5.5 vs.10.5 µg/ml), C_{min} (2.4 vs.6.2 µg/ml) and AUC_{0-24} (78.4 vs.176.4 µg/ml.h) than those with body weight $\leq$ 50 kg (n=20); these differences were not statistically significant. C_{max}, C_{min} and AUC were significantly higher in TT genotypes than GT and GG genotypes ($p < 0.05$). Rifampicin co-administration caused significant reduction in C_{max}, C_{min} and AUC_{0-24} ($p < 0.05$), the mean percent decreases being 26%, 29% and 25% respectively (Table 5.6). Three out of 15 patients had C_{min} below sub-therapeutic level (<1 µg/ml) in the presence of R.

Conclusions:

CYP2B6 G516T polymorphism and R co-administration significantly influence the pharmacokinetics of EFV. Although R co-administration and higher body weight caused reduction in plasma EFV concentrations, dose enhancement of EFV may not be necessary since the C_{min} of EFV was above 1 µg/ml, that is, the drug concentrations did not drop to sub-therapeutic levels in majority of the patients studied.

Table 5.6: Steady state pharmacokinetics of EFV with and without R

Patients n=15	C_{max}	C_{min}	T_{max}	$AUC_{(0-24)}$	Cl	$T_{1/2}$
EFV	9.33 ± 8.04	5.28 ± 6.33	2.60 ± 1.06	156.04 ± 174.72	6.62 ± 4.12	24.98 ± 13.19
EFV + R	6.86 ± 6.52	3.76 ± 5.21	2.47 ± 0.99	116.52 ± 135.69	10.26 ± 6.08	17.57 ± 9.42
% decrease	26.5%	28.8%	--	25.3%	55.0%*	--
p value	<0.05	<0.05		<0.05	<0.05	<0.05

*percent increase;
C_{max} – Peak concentration; C_{min} – Trough concentration; T_{max} – Time to attain C_{max}; $AUC_{(0-24)}$ – Exposure; Cl – Clearance; $t_{1/2}$ – Half-life

(PI: Dr. Geetha Ramachandran; geethar@trcchennai.in)

Ongoing Studies:

Detection of mycobacterial species by high performance liquid chromatography

Background:

Identification of mycobacteria has traditionally been based on growth characteristics and a battery of time-consuming biochemical tests. Unfortunately, the specificity and sensitivity of these tests in recognizing new species are low. Only genetic and chromatographic analyses seem to have the potential to recognize new species of mycobacteria. Reverse phase-HPLC of high molecular weight mycolic acids are rapid and unlike genetic probes are not limited to the identification of only a few species. Non-tuberculous mycobacteria (NTM) have been reported to cause localized or disseminated disease especially in HIV patients. This was particularly true in case of *M.avium* complex (MAC), because these organisms are the single most important cause for disseminated bacterial infection in AIDS patients.

Aims:

- To identify mycobacterial species from primary culture isolates by HPLC
- To assess the prevalence of NTM species among these isolates

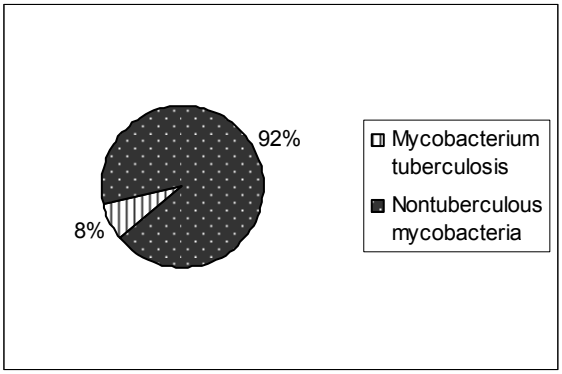
Methods:

Sputum samples referred to TRC during the year 2006 were included in the study. The cultures with atypical morphology or cultures with discordant results by biochemical tests were subjected to HPLC for species level identification. The parameters set for the HPLC system, chemicals and solvents, testing procedures were followed as per Centers for Disease Control (CDC), USA protocol. A set of standard strains were processed along with the specimens and used as controls for chromatographic pattern comparison.

Results:

Out of 212 isolates, 179 were subjected for species level identification by HPLC among which 165 (92%) were identified as NTM and 14 (8%) as *M. tuberculosis* (Fig. 5.6). The predominant NTM species were found to be *M.kansasii* (28%), followed by *M.avium* (15%), *M.fortuitum* complex (12%), *M.intracellulare* (9%) and *M.parafortuitum* (8%).

Fig.5.6: Results of HPLC analysis



(PI: Dr.Ranjani Ramachandran; ranjanir@trcchennai.in)

Innate and adaptive immunity in children starting antiretroviral drugs in India

Background:

Globally, more than 2000 children acquire HIV infection daily from their mothers, 90% of whom reside in developing countries. Despite the well-known benefits of ART for the treatment of AIDS, access to therapy has been a problem for the majority of HIV-infected children in the world. In India, the government has recently initiated a program to provide free ART to children below 15 years of age. There is a need to gather pediatric specific clinical data and laboratory indicators of the risk of progression during the first few years of life.

Aim:

- To identify novel laboratory markers that provide insights into functional immune status of HIV-infected children that are better than assays of CD4 positive T cells and plasma viral load.

Methods:

This project will enroll HIV infected children (birth -13 years) who will be given ART as per standard of care based on national guidelines of India and who will be monitored for immunologic and virologic status by routine and proposed novel markers of innate and adaptive immunity. These assays include studies of dendritic cell number and function and surface markers on CD4 and CD8 positive T cells. Patient enrollment to this study started in February 2007. So far, 23 children have been enrolled into the study.

(PI: Dr. Soumya Swaminathan, : soumyas@tgrcchennai.in; Funding: Indo-US JWG Maternal and Child Health (NIH and ICMR)

Therapeutic drug monitoring of nevirapine and efavirenz in HIV-infected children in India

Background:

Highly active antiretroviral therapy has resulted in an impressive reduction in the rate of disease progression and death in HIV-1 infected children. Antiretroviral drugs for pediatric use have been very recently introduced by the Government of India and are made available at the Government ART centres. Limited information is available on the blood levels of NVP and EFV in children receiving the pediatric formulations in India.

Aim:

- To estimate the trough and peak levels of NVP in HIV-infected children receiving treatment with generic pediatric formulations and examine if drug levels are within the therapeutic range of the drugs (In the case of children receiving EFV-based regimens, the 12-hour drug level will be studied)

Methods:

HIV-infected children receiving treatment at the Government ART centres at the Government Rajaji Hospital, Madurai, B.J.Wadia Hospital, Mumbai, Government Hospital of Thoracic Medicine, Tambaram and Kilpauk Medical College and Hospital, Chennai will form the study population. Children aged 6 months to 14 years, receiving treatment regularly with generic antiretroviral drugs as fixed dose combinations (FDCs) based on body weight/body surface area, for at least two weeks will be recruited. It has been proposed to include about 100 children in the study. Blood samples before and at two hours after drug administration will be collected. In the case of EFV, a single blood sample will be collected. Plasma NVP and EFV estimations will be carried out by HPLC. The blood levels of NVP and EFV obtained in children will be examined to see if they fall within the therapeutic range of the drugs (3 – 12 µg/ml for NVP and 1 – 4 µg/ml for EFV).

So far, 58 and 11 children receiving NVP and EFV respectively have been included into the study. The study is in progress.

(PI: Dr. Soumya Swaminathan; : soumyas@trcchennai.in)

Feasibility of monitoring antiretroviral treatment adherence using urine and salivary lamivudine: a study in healthy volunteers

Background:

Adherence to ART is a strong predictor of virological suppression, disease progression and death. Although currently available approaches to measure

adherence have proved to be predictive of outcomes, the results are variable. Some investigators have assessed the antiretroviral drug levels in blood as a measure of adherence. Alternatively, urine or saliva could serve as useful biological fluids for detecting antiretroviral drug levels particularly to monitor patient adherence to treatment if found feasible. Lamivudine, a cytosine nucleoside analogue has been effectively used in combination with other antiretroviral drugs to treat HIV-1 infection. Its levels in urine or saliva could be used to study patient adherence to treatment.

Aims:

- To estimate the concentration of 3TC excreted in urine collected at different time points between 0 – 24 hours of drug administration
- To estimate the concentration of 3TC in paired plasma and saliva collected at different time points after drug administration

Methods:

The study is being carried out in collaboration with Department of Pharmacology, Madras Medical College, Chennai. Twelve healthy male volunteers will take part in the study. On the day of the study, a sample of blood (three ml) will be collected in a heparinised vacutainer. One tablet of 3TC (150 mg) will be administered with 200 ml water. Blood samples will be collected at 1, 2, 4, 6, 8, 12 and 24 hours of drug administration. They will be instructed to collect about 3 ml of saliva at the same time points. Complete urine collections made between 0-8, 8-16 and 16-24 hours will be collected in separate containers. Lamivudine concentrations in plasma, saliva and urine will be estimated by HPLC. Based on plasma and salivary concentrations of 3TC at different time points, certain pharmacokinetic variables will be calculated. The percent dose of 3TC excreted in urine collected at different time periods will be calculated.

So far, eight subjects have been recruited into the study. The study is in progress.

(PI: Dr. Geetha Ramachandran; geethar@trcchennai.in)

Multicentric evaluation of a sensitive smear microscopy technique for detection of AFB in sputum

Background:

The smear method is essentially a component of a multipurpose method that is applicable on both pulmonary and extra-pulmonary specimens and also compatible with culture and PCR techniques. This method is likely to identify a significant number of patients who go undetected by the direct method of smear microscopy thereby contributing to improved disease control.

Aim:

- To evaluate a highly sensitive method of smear microscopy developed in Dr. Jaya S. Tyagi's laboratory at All India Institute of Medical Sciences, New Delhi in a multicentric laboratory setting

Methods:

Sputum samples collected from Otteri TB Hospital are transported to the TRC Bacteriology laboratory and processed within 4 to 6 hours. A maximum of 8 – 10 samples are processed per day. After receiving the sputum sample direct smear is made. Later each sample is divided into two portions of approximately equal volume. One portion is processed by modified Petroff's method and the other portion by the universal sample processing (USP) method for culture. The intake is continuing and about 800 samples are processed.

(PI: Dr.N.Selvakumar; : selvakumarn@trcchennai.org. Funding: WHO/ICMR)

Standardization of second line anti-TB drug susceptibility testing

Background:

The global situation with respect to TB has worsened with the emergence of MDR-TB. Various studies have shown that MDR-TB can be cured by a combination of second-line drugs under DOTS-Plus. With the spread of MDR-TB, there is an increasing demand for DST for second line drugs. Since the critical concentration for second line drugs have not been completely established, the present study is focusing on evaluating the critical concentration of some of the second line drugs namely kanamycin (K), ethionamide (Eth), capreomycin (C), amikacin (A), PAS and Z. Previously established critical concentrations for

second line drugs using conventional methods will be performed and compared with automated systems and other rapid phenotypic methods. After establishing the critical concentrations, the results will be analyzed for overall correlation between the various methods used.

Aims:

- To standardize DST of second line drugs using standard conventional methods and automated systems and other rapid phenotypic methods
- To compare the results obtained by automated and phenotypic rapid methods with that of standard conventional methods

Methods:

Clinical isolates from the patients attending the TRC clinic will be selected for this experiment. Selection of isolates are based on their first line drug susceptibility pattern, where 50% of them are MDR and 25% were polyresistant to first line drugs and 25% fully susceptible to all drugs. Isolates freshly subcultured onto LJ medium will be used for setting up DST after randomization. The following methods for DST will be used viz: LJ, agar, liquid medium, automated systems and colorimetric methods.

(PI: Dr.Ranjani Ramachandran : ranjanir@trcchennai.in Funding: WHO-USAID)

Microscopic observation drug susceptibility assay as a rapid low cost test for detection and drug susceptibility of *Mycobacterium tuberculosis* in HIV-TB and non HIV-TB Individuals

Background:

In the last decade, there has been dramatic resurgence in the incidence of TB throughout the world. The situation is compounded with increasing HIV infections. India is estimated to have 3.5 million HIV patients and about 1.8 million of them are co-infected with TB. Most cases occur in developing, resource poor countries, where the expensive automated systems (MGIT 960 and BACTEC 460) are not feasible. To curb the transmission of infection early detection of MDR-TB using a low cost rapid test is the need of the hour. The principle of the microscopic observation broth–drug susceptibility (MODS) test is

based on the microscopic detection of *M. tuberculosis* as strings or cords in Middlebrook 7H9 broth medium with or without antimicrobial agents.

Aims:

- To evaluate MODS for the detection and drug susceptibility of *M. tuberculosis* in sputum samples from patients with suspected pulmonary TB in HIV positive and non-HIV individuals
- To compare the results of MODS with conventional methods and automated systems to detect cross contamination and relative cost

Methods:

The sputum samples referred to TRC will be processed for AFB culture as per standard protocol. The remaining sputum sediment will be inoculated into 7H9 broth with and without drug and periodically examined for cord formation under inverted microscope. DST is performed for the following drugs: Sm, H, R, E, K, Eth and ofloxacin (O). The results of MODS after decoding will be subjected to statistical analysis.

(PI: Dr.Ranjani Ramachandran : ranjanir@trcchennai.in Funding: WHO-USAID)

Tetrazolium micro plate assay - Rapid colorimetric method and thin layer agar for determination of DST of *M. tuberculosis*

Background:

Tuberculosis has acquired a growing importance in developed and developing countries with the emergence of MDR-TB. To control the spread of MDR-TB rapid and reliable method for early diagnosis and rapid DST is the need of the hour. There are various rapid methods available for DST, which includes, automated system using BACTEC 460 and MGIT 960 (Becton and Dickenson) and molecular methods such as INNO-LiPa (Line probe assay) that are expensive and are impractical for routine use. In the last few years much attention has been posed towards colorimetric assays such as NRA using solid media, micro alamar blue assay (MABA) and tetrazolium microplate assay (TEMA), which are quite rapid and cost effective and easy to perform.

Aims:

- To standardize and evaluate TEMA and thin layer agar (TLA)
- To determine susceptibility of *M.tuberculosis* for first and second line drugs
- To compare the TEMA and TLA results with that of conventional methods

Methods:

Samples size:

One hundred and thirty cultures from patients referred to TRC were selected for the study and randomized by statistician.

TEMA: A rapid colorimetric method based on the principle of reduction of MTT [3-(4,5-dimethyl thiazol-2-yl)-2,5 diphenyl tetrazolium bromide] for determination of susceptibility to the anti-TB drugs (Sm, H, R, E, K, Eth and O).

Culture suspension was prepared in 7H9 GC broth and tested for drug susceptibility using 96 well microtitre plate for the above mentioned anti-TB drugs. After incubation readings will be taken on days 5, 7, 9, 11, 13 and 15 respectively. Resistance is determined by the development of purple colour and susceptibility by no colour change in the appropriate drug concentrations.

TLA: This method is capable of detecting growth within 9-11 days. Initial identification of *M. tuberculosis* based on colony morphology in solid medium when viewed microscopically can be assessed.

Culture suspension prepared in 7H9 broth and 10 fold dilutions will be prepared (S1, S2, S3 & S4). Drug susceptibility testing is performed for the following drugs (Sm, H, R, E, K, Eth and O).

After incubation readings will be taken on days 3, 5, 7 and10 respectively under an inverted light microscope. Susceptibility pattern can be determined by detecting microcolonies in drug free and drug containing plates. Percentage of resistance is calculated. After completion of the experiments, the strains will be decoded and results will be analyzed using appropriate statistical method.

(PI: Dr.Ranjani Ramachandran : ranjanir@trcchennai.in Funding: WHO-USAID)

6. BASIC RESEARCH

Completed Studies:

Development of luciferase reporter phages aiding diagnosis of latent tuberculosis

Background:

Early diagnosis of TB is essential for reducing the morbidity and mortality both in endemic and in HIV infected population. LRPs show promise in diagnostic microbiology. LRP constructs from D29 and TM4 used in conventional LRP assay are highly specific but lack in sensitivity as the lytic phage infection proceeds to host cell lysis. It was hypothesized that a luciferase reporter construct from temperate phage infecting *M. tuberculosis* would bring about a sustained light output leading to better sensitivity of LRP assay. Most of the TB cases among the HIV-infected population result from the reactivation of latent bacilli. LRP constructed with a dormancy inducible promoter driving the luciferase gene must be able to detect viable but not cultivable population of *M. tuberculosis* in clinical specimens.

Aim:

- To evaluate the performance of LRPs developed from temperate mycobacteriophage Che12 and TM4 derived temperature sensitive (TS) mutant, phAE159, with the conditional promoters, namely isocitrate lyase (icl) and alpha crystallin protein (acr)

Methods:

Light production kinetics of the LRP constructs was studied in *M. tuberculosis* H_{37}Rv. Luciferase activity of the LRP constructs was evaluated and compared with D29 based LRP phAE129 in both active and dormant (Wayne's dormant model) cultures of *M. tuberculosis* H_{37}Rv and five clinical isolates of *M. tuberculosis.*

Results:

TM4 based phAETRC201 expressing firefly luciferase gene driven by hsp60 promoter and phAETRC202 with 'acr' promoter showed peak activity at 48 hours

(Figure 6.1). In the case of the Che12 construct phAETRC21 with 'icl' promoter, RLU reached maximum at 72 hours (Figure 6.2). All the three constructs exhibited detectable luciferase activity in actively growing cultures of *M. tuberculosis*. With the Wayne's dormant model phAETRC201 gave detectable light output with an increase in RLU by more than one log in all the strains tested, whereas phAETRC202 gave detectable RLU with only 4 of the isolates. PhAE129 gave detectable RLU in 3 strains tested (Figure 6.3). One of the clinical isolates resulted in detectable RLU only with phAETRC201 (TM4 based) and this was subsequently identified as a NTM.

Fig. 6.1: Light kinetics of TS mutant LRP constructs in *M.tuberculosis* $H_{37}Rv$

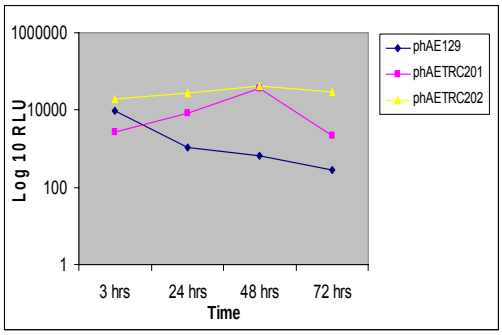


Fig. 6.2: Light kinetics of Che12 constructs in *M.tuberculosis* $H_{37}RV$

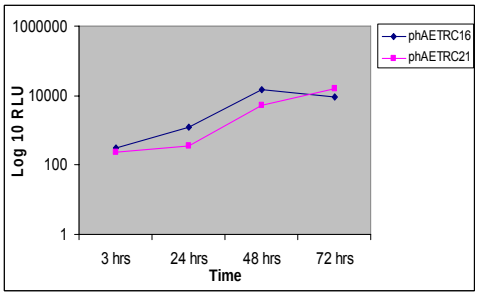
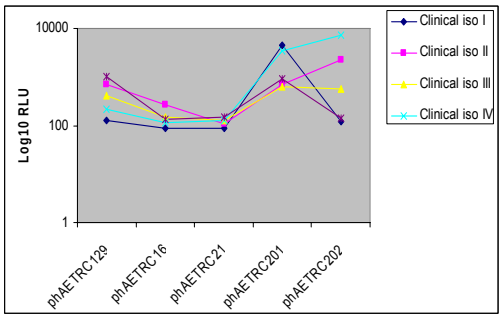


Fig. 6.3: Comparison of different LRPs in dormant cultures after 24 hours infection



Conclusion:

As the constructs exhibited detectable RLU at varying levels both in active and dormant models, it is recommended that use of all these constructs together should yield best results in TB diagnosis. Refining the assay format further should lead to an ideal assay to diagnose TB, to differentiate the species and to do direct DST.

(PI: Dr.Vanaja Kumar, vanajakumar@trcchennai.in)

The status of the complement system in tuberculosis

Background:

The complement system serves as a major link between the innate and the adaptive immune system as well as facilitating production of appropriate immune response to an antigenic stimulus. It is known that mycobacteria and some of their antigens activate the complement system and the complement components are found in circulating immune complexes (CIC) in active TB. Further, levels of complement proteins and hemolytic complement have been shown to be higher in patients compared to healthy controls. Mice deficient in complement components are more susceptible to *M. tuberculosis* infection than control mice. These findings suggest that the complement system is involved in the pathogenesis of TB.

Aim:

- To investigate the status of the complement system in TB by documenting the quantitative changes that occur in this disease, functional aspects of the complement system and the effect of complement components on the host immune responses in pulmonary TB

Methods:

The levels of complement components and activation fragments in serum and complement receptors on peripheral blood mononuclear cells (PBMCs) were measured. The alteration of the functional capacity of the activated complement system in TB and the effect of complement on the immune responses against *M. tuberculosis* was studied.

Participants:

The study subjects comprised of 25 patients each with active, smear positive pulmonary TB and 25 patients who have completed the entire ATT regimen and 25 normal healthy volunteers.

Complement profile in TB:

The levels of complement components and their activation fragments were measured in serum using sandwich ELISA and the surface expression of complement receptors on PBMCs using flow cytometry.

Functional assay:

Functional characterization of complement activation was assessed by ELISA using coated IgM for the classical pathway and cell surface glycoproteins of mycobacteria for the alternative pathway.

Effect of the complement system on host immune response

The functional significance of the activated complement system on important host immune responses against *M. tuberculosis* was studied by analyzing the levels and components of CICs using ELISA, and the effect of *in vitro* addition of complement components on macrophage phagocytosis and apoptosis of mononuclear cells using flow cytometry.

Results:

Complement activation fragments like C3a, C4a, C5a and C3d were found to be higher in active TB patients followed by post treatment and control groups (Table 6.1).

Table 6.1: Serum complement profile showing the levels of C3, C3d, C3a, C4, C4a, factor B and C5a measured using ELISA (mean ± 95% CI).

	NHC (n = 25)	Treated TB (n = 25)	Active TB (n = 25)
C3 (AU/ml)	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.04
C3d (AU/ml) *	0.6 ± 0.1	1.0 ± 0.1 [a]	1.3 ± 0.1 [b, c]
C3a (ng/ml) *	11712.3 ± 2319.5	12949.7 ± 1948.8	21845.4 ± 1572.1 [b, c]
C4 (AU/ml)	0.9 ± 0.1	0.9 ± 0.2	0.9 ± 0.04
C4a (ng/ml) *	1008.6 ± 452.6	1412.4 ± 576.7	2236.7 ± 244.5 [c +, b $]
Factor B (AU/ml)	0.8 ± 0.2	0.6 ± 0.2	0.8 ± 0.1
C5a (ng/ml) *	153.8 ± 50.3	691.7 ±192.4 [a]	1175.3 ± 253.8 [b, c £]

NHC-normal healthy controls; TR-treated PTB patients; UTR-untreated active PTB patients; n-number of individuals; CI-confidence interval

* Statistically significant differences (P<0.005) between groups (ANOVA).
a Differences are significant between NHC and TR groups (Post Hoc Bonferroni test).
b Differences are significant between NHC and UTR groups (Post Hoc Bonferroni test).
c Differences are significant between TR and UTR groups (Post Hoc Bonferroni test).
# P<0.001, $ P=0.001, + P=0.038, £ P=0.002 by Post Hoc Bonferroni test.

The levels of native complement proteins were comparable amongst the three study groups. However, high levels of complement split fragments were seen in patients compared to controls.

Under *in vitro* condition, the presence of complement is shown to modulate cytokine secretion by monocytes infected with *M. tuberculosis*.

Analysis of the components of CIC in pulmonary TB showed that the presence of complement components like C3d, C4c and C3 and the extent of immune complex precipitation was found to be higher in active TB patients compared to the other two groups examined.

Decreased expression of all four complement receptors on various cell types with different surface markers such as CD4, CD8, CD20 and CD14 was observed.

CR1 on erythrocytes were found to be decreased on erythrocytes of patients with active TB compared to treated patients and controls.

Conclusion:

The presence of complement is shown to augment the percentage of mononuclear cells undergoing apoptosis infected with *M. tuberculosis*. The complement system is greatly perturbed in active pulmonary TB. Addition of complement can augment the production of key cytokines and also increase apoptosis induced by *M.tuberculosis*.

(Dr.V.D. Ramanathan, vdramanathan@trcchennai.in)

Evaluation of humoral immune response against ESAT- 6 and ESAT- 6:CFP - 10 polyprotein in diagnosis of tuberculosis

Background:

Development of immunoassays specific for diagnosis of TB requires antigens unique to *M. tuberculosis.* Early secretory antigen target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) are the two immunodominant antigens encoded by region of difference (RD)-1, which are present only in *M. tuberculosis* complex, but not in BCG. Using CFP-10 alone, we could obtain reasonably good sensitivity in TB cases. This interesting observation provided the impetus for working on its dimeric coprotein ESAT-6 and polyprotein (comprising of ESAT-6 and CFP-10).

Aim:

- To study the ability of species-specific antigen ESAT-6 and ESAT-6:CFP - 10 polyprotein to enhance sensitivity, when combined with the species-specific antigen 38kDa

Methods:

Enzyme linked immunosorbent assay (ELISA) for antibody estimation (IgG and IgA) was carried out in the following groups:

Disease groups:

HIV seronegative tuberculosis (n=319):

1. Smear and culture positive patients with pulmonary TB (S+C+) (n=142)
2. Smear negative and culture positive patients (S-C+) with pulmonary TB (n=35)
3. Smear and culture negative, but radiologically diagnosed cases (S-C-) (n=142)

HIV-TB coinfected subjects (n=138):

4. S+C+ (smear and culture positive) (n=77)
5. S-C+ (smear negative and culture positive) (n=43)
6. S-C- (smear negative culture negative) (n=18).

Control groups (n=201):

7. Normal healthy subjects (NHS) (n=126)
8. HIV infected subjects (n=75)

Results:

The levels of IgG and IgA were measured individually and the results were combined for analysis. The cut-off value for each antigen was determined using mean O.D. + 3 SD of NHS.

The results are given in Table 6.2**.** While combining results of both the isotypes the ESAT-6 showed sensitivities of 18%, 24% and 54% and polyprotein showed 6%, 23% and 24% in S+C+, S-C+ and S-C- cases. ESAT-6 and polyprotein both showed more than 95% specificities.

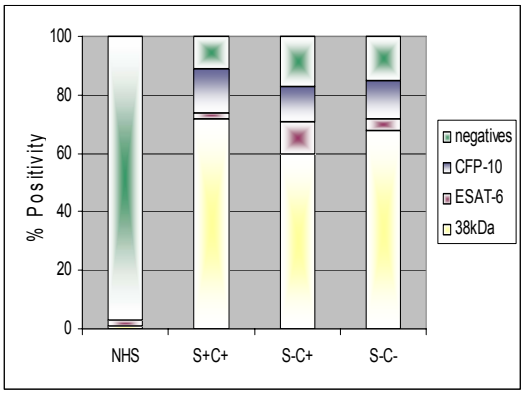
Table 6.2: Antibody profiles of ESAT-6 and Polyprotein

Antigens	Subjects	Number of samples tested	Number of samples detected		
			IgG	IgA	IgG+IgA
ESAT-6	S+C+	113	8(7)	16(14)	20(18)
	S-C+	35	5(14)	11(31)	12(24)
	S-C-	136	29(21)	63(46)	73(54)
	NHS	124	1(1)	2(2)	3(2)
Polyprotein	S+C+	113	7(6)	0(0)	7(6)
	S-C+	35	8(23)	1(2)	8(23)
	S-C-	136	24(18)	21(15)	32(24)
	NHS	124	2(2)	3(2)	4(3)

NHS Normal healthy subjects
S+C+ Smear positive and culture positive PTB patients
S-C+ Smear negative and culture positive PTB patients
S-C- Smear negative and culture negative PTB patients

The combination of 38kDa, CFP-10 and ESAT-6 showed sensitivities of 89%, 83% and 85% in S+C+, S-C+ and S-C- cases with the specificity of 97% (Fig. 6.4).

Fig.6.4: Combination of 38kDa, CFP-10 and ESAT-6



NHS	Normal healthy subjects
S+C+	Smear positive and culture positive PTB patients
S-C+	Smear negative and culture positive PTB patients
S-C-	Smear negative and culture negative PTB patients

Similar analysis of results obtained with the HIV-TB group showed that using ESAT-6 antigen alone or ESAT-6; CFP-10 polyprotein has lower utility in patients infected with HIV.

Conclusion:

Combination of ESAT-6 with 38kDa and CFP-10 improves the sensitivity by 14% in S-C+ cases, where the diagnosis is the most needed.

(PI: Dr. Alamelu Raja, alamelur@trcchennai.in)

Influence of HLA-DRB1 alleles on cytokine response to *M. tuberculosis* and its culture filtrate antigen in pulmonary tuberculosis

Background:

Our earlier studies revealed that HLA-DR antigens can influence humoral and cell mediated immune response to TB. Cytokines play a central role in immune response to *M. tuberculosis* infection. Specific antigen presentation to CD4+ T cells and HLA-DR might modulate cytokine response to *M. tuberculosis* infection.

Aim:

- To study the influence of HLA-DRB1 alleles on live *M. tuberculosis* and its culture filtrate antigen (CFA) induced cytokine response by measuring Th1

(IFN-γ & IL-12p40), Th2 (IL-4 & IL-5), pro-inflammatory (IL-6 & IL-8) and anti-inflammatory (TGF-β & IL-10) cytokines in healthy controls and newly diagnosed PTB patients

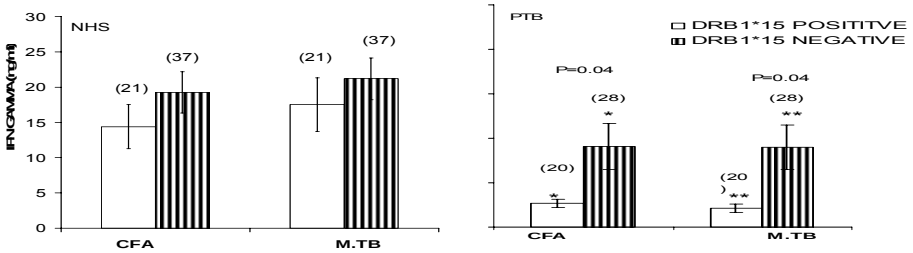
Methods:

Study subjects consisted of 58 NHS and 48 PTB patients. Cytokine levels of IFN-γ, IL-12p40, IL-4, IL-5, IL-6, IL-8, IL-10 and TGF-β in the culture supernatants of PBMC stimulated with *M.tuberculosis* and its CFA, were measured using commercial ELISA kits. HLA-DRB1 genotyping was carried out by DNA based PCR and dot blot hybridization with allele specific oligonucleotide probes and detection by chemiluminescence method.

Results:

Pulmonary TB patients showed significantly increased levels of IL-6, IL-8 and TGF-β and decreased levels of IFN-γ, IL-12p40 and IL-10 in response to live *M.tuberculosis* and its CFA as compared to NHS. Patients with HLA-DRB1*15 allele exhibited a significantly lower IFN-γ response compared to respective allele negative patients (p=0.04) (Fig. 6.5).

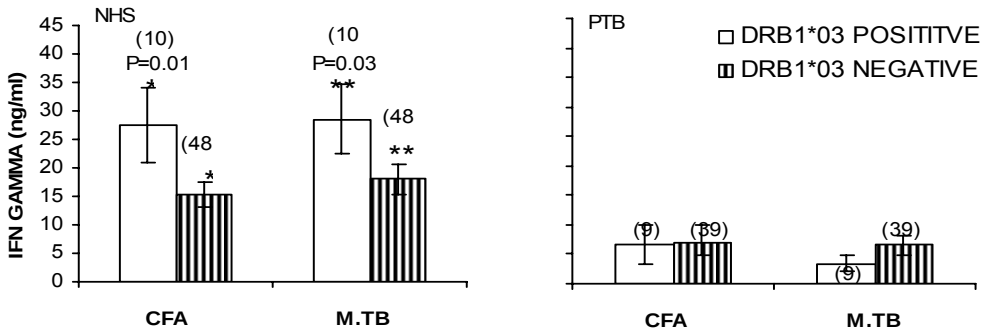
Fig-6.5: CFA and live *M. tuberculosis* induced IFN-γ level in HLA-DRB1*15 positive and negative NHS and PTB patients



An increased IFN-γ response was observed in HLA-DRB1*03 compared to respective allele negative NHS (p=0.03) when stimulated with live *M. tuberculosis* and culture filtrate antigen (Fig. 6.6). An increased level of IL-12p40 was observed in DRB1*10 (p=0.02) and IL-10 in DRB1*12 (p=0.03) positive NHS and an increased level of IL-6 in DRB1*04 (p=0.02) positive PTB patients, compared

to respective allele negative individuals. HLA-DRB1 alleles did not modulate IL-4 and IL-5 response to *M. tuberculosis* and its CFA.

Fig. 6.6: CFA and live *M. tuberculosis* induced IFN-γ level in HLA-DRB1*03 positive and negative NHS and PTB patients



Conclusion:

The study revealed that HLA-DRB1 alleles differentially modulate Th1 cytokines namely IFN-γ and IL-12p40, anti-inflammatory cytokine IL-10 and pro-inflammatory cytokine IL-6 in response to *M*. *tuberculosis* and its antigens. This suggests that HLA-DRB1 alleles might modulate the immune response to *M. tuberculosis* infection by altering cytokine production.

(PI: Dr. P. Selvaraj; selvarajp@trcchennai.in)

Regulatory role of variant genotypes of vitamin-D receptor gene on cytokine response in pulmonary tuberculosis

Background:

Our earlier studies revealed the immunomodulatory effects of vitamin D_3 on macrophage phagocytosis and lymphocyte functions in TB. Studying the influence of vitamin D_3 on mycobacterial antigen induced cytokine response will help in understanding the basic molecular events associated with pulmonary TB.

Aim:

- To study the regulatory role of vitamin D receptor gene variants on vitamin D_3 modulated Th1 and Th2 cytokine production in response to *M.tuberculosis* antigens in pulmonary TB

Methods:

Peripheral blood mononuclear cells isolated from 60 active PTB patients and 65 NHS were cultured with live *M.tuberculosis* $H_{37}Rv$ and its culture filtrate antigen in the presence and absence of vitamin D_3 and maintained for 72 hours at 37° C and 5% CO2. The cytokines IL-8, IL-6, IFN-γ, IL-12p40, IL-2, IL-10, TGF, IL-4 and IL-5 were estimated in the culture supernatants by commercially available ELISA kits.

Genotyping of vitamin D receptor (VDR) gene was done for *Apa* 1, *Bsm* 1 , *Taq* 1 and *Fok* 1 polymorphisms by PCR-RFLP.

Results:

In normal healthy subjects, vitamin D_3 significantly suppressed IL-12p40 and IFN-γ production in response to CFA and live *M.tuberculosis* in a dose dependent manner with a maximum suppression at 10^{-7}M concentration ($p<0.0001$). In patients, a significant dose dependent decrease in IL-12p40 levels in response to CFA (10^{-7}M, $p<0.0001$) and live *M. tuberculosis* (10^{-7}M, $p <0.0001$) (Fig. 6.7) and a significant decrease in IFN-γ production in response to CFA (10^{-7}M, $p=0.01$) was observed. In healthy controls, the IL-2 levels were increased upon vitamin D_3 addition in CFA stimulated cultures, but no effect was seen in patients (Figure 6.8 a&b). Addition of vitamin D_3 showed an increased trend for IL-4 and a decreasing trend for IL-5 levels.

Fig. 6.7 – Effect of Vitamin D_3 on IL-12 p40 levels in NHS and PTB

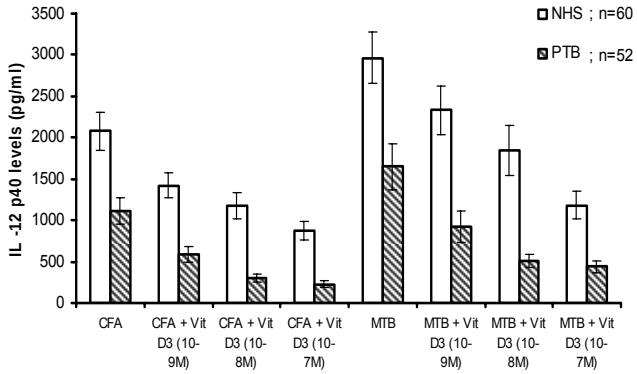
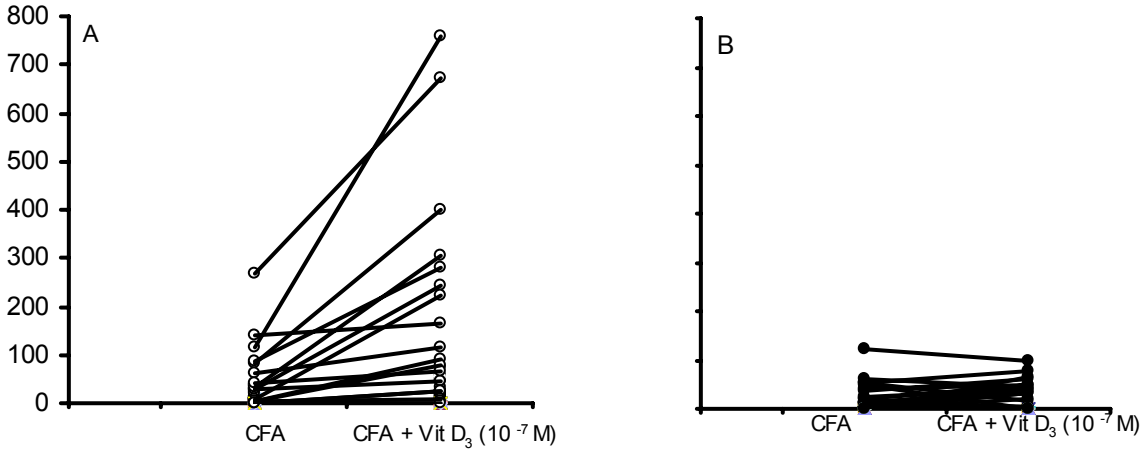


Fig.6.8a&b: a) Effect of vitamin D_3 on CFA induced IL-2 in NHS
b) Effect of vitamin D_3 on CFA induced IL-2 PTB



The cytokine levels were further correlated with the vitamin D receptor genotypes to study the regulatory role of VDR gene variants on vitamin D_3 modulated cytokine production. In CFA stimulated cultures of NHS, the IFN-γ level was increased in 'tt' genotype when compared to TT genotype. The IL-10 production was significantly increased in *M.tuberculosis* and vitamin D_3 treated cultures (1×10^{-9} M; p=0.05, 1×10^{-8} M and 1×10^{-7} M; p=0.01) in TT genotype when compared to 'tt' genotype in NHS. In PTB, the IL-10 levels were slightly increased in patients with 'tt' genotype, but it was not statistically significant.

Conclusions:

The present study suggests that vitamin D_3 exerts differential effects on cytokine production in TB with a prominent suppressive effect on IL-12p40 and IFN-γ and that the variant VDR genotypes regulate the vitamin D_3 modulated cytokine response.

(PI: Dr. P. Selvaraj; selvarajp@trcchennai.in)

Human leucocyte antigen (HLA) and non-HLA gene polymorphism studies in HIV and HIV-TB patients

Background:

In developing nations, HIV-1 infection has increased the burden of TB especially in populations where the prevalence of TB infection is high among young adults. The importance of host genetic factors (HLA and non-HLA) on susceptibility or resistance to HIV infection and the variability in disease progression towards AIDS has been emphasized by many studies.

Aim:

- To find out whether polymorphisms in Human Leucocyte Antigens (HLA) – A, –B, -DR and –DQ antigens and non-HLA genes (Mannose binding lectin gene) are associated with susceptibility or resistance to HIV and HIV-TB

Methods:

The study subjects include 151 HIV positive TB negative patients (HIV+TB-), 117 HIV positive TB positive patients (HIV+TB+), 162 HIV negative TB positive patients (HIV-TB+) and 186 healthy controls. HLA –A, –B antigens were typed using microlymphocytotoxicity assay and -DR and –DQ typing was done by PCR with locus specific primers followed by hybridization with allele specific oligonucleotide probes (ASOP) and detection by chemiluminescence method. Mannose binding lectin-2 (MBL-2) structural genotypes (54, 57 and 52) were identified using PCR-ASOP. Promoter genotypes (-221 X/Y) were identified using PCR with allele specific primers. MBL levels from plasma were estimated using an MBL oligomer ELISA kit.

Results:

Highly decreased frequency of HLA –A11 was observed in HIV patients with and without TB compared to controls (Controls vs Total HIV: Pc=0.00044; Controls vs HIV+TB-: p=0.021; Controls vs HIV+TB+: Pc=0.0001). Significantly increased frequency of HLA –B40 was noticed in HIV patients with and without TB compared to controls (Controls vs Total HIV: Pc=0.014; Controls vs HIV+TB- p=0.011; Controls vs HIV+TB+: Pc=0.017). Increased frequency of HLA –DR2

was noticed in HIV patients with and without TB compared to controls (Controls vs Total HIV: Pc=0.024; Controls vs HIV+TB- : Pc=0.024; Controls vs HIV+TB+ p=0.036). Significant increase of HLA –DR2 was also observed in HIV-TB+ as compared to controls (Controls vs HIV-TB+ Pc=0.043) (Table 6.3). Data on DQB1 typing is being analysed.

Table 6.3: Percent frequencies of selected HLA antigens among healthy controls, HIV patients without and with TB and HIV negative patients with TB

HLA antigens	Healthy controls (n=186) %F	Total HIV infected patients (n=268) %F	HIV+TB- (n=151) %F	HIV+ TB+ (n=117) %F	HIV-TB+ (n=162) (%F)
A11	28.5(53)	12.7(34)	17.2(26)	6.8(8)	24.7(40)
B40	21.0(39)	36.2(97)	33.8(51)	39.3(46)	31.5(51)
DR2	34.3(47)	49.4(124)	53.2(74)	48.5(50)	51.9(80)

For DR, n=137 for controls, n=139 for HIV+TB-, n=103 for HIV+TB+, n=154 for HIV-TB+.
Numbers in the parentheses represent individuals positive for that genotype
n= number of subjects studied, F= antigen frequency.

Plasma MBL levels were significantly higher in HIV+TB+ patients compared to HIV+TB- patients. A significantly increased frequency of YA/YA diplotype (associated with high MBL levels) was observed among HIV patients with TB compared to controls (p=0.0108). A significantly increased frequency of YO/YO diplotype (associated with low MBL levels) was observed among HIV negative TB patients than in controls (p=0.036) (Table 6.4).

Table 6.4: Percent frequencies of selected MBL diplotypes among healthy controls, HIV patients without and with TB and HIV negative patients with TB

Diplotypes	Healthy controls (n=146) %F	HIV+TB- (n=151) %F	HIV+ TB+ (n=112) %F	HIV-TB+ (n=146) (%F)
YA/YA	19.2 (28)	23.2 (35)	33.9 (38)	26.7 (39)
YO/YO	4.8 (7)	8.6 (13)	6.2 (7)	12.3 (18)

Numbers in the parentheses represent individuals positive for that genotype
n= number of subjects studied, F= Diplotype frequency

Conclusion:

The study suggests that HLA –A11 may be associated with resistance to HIV infection and HIV with active TB, while HLA –B40 and HLA –DR2 may be associated with susceptibility to HIV and HIV with active TB. This finding may have implications for vaccine design and to develop effective prophylactic strategies against HIV and TB. High MBL levels and diplotypes associated with increased MBL levels might have a predisposing effect on the development of TB in HIV infected individuals and diplotypes associated with low MBL levels may be a risk factor for development of TB in HIV negative individuals.

(PI: Dr. P. Selvaraj; : selvarajp@trcchennai.in Funding: ICMR Task Force)

Human monocyte and macrophage apoptosis induced by *M.tuberculosis* strains and its implication on cell mediated immune response

Background:

Apoptosis is an orchestrated suicide program, which enables macrophages to remove invading pathogens without inducing an inflammatory response. The regulation of programmed cell death is a complex process involving a myriad of proteins within the cell. In TB, the reduction in bacillary load is associated with apoptotic death of infected macrophages at the site of infection. The ability of *M. tuberculosis* to survive within the macrophage for an extended period is its

greatest success. Hence it is important to understand the apoptotic signals and survival strategies of the *M. tuberculosis* to offset cell death for pathogenesis. Our previous findings suggested that two prevalent clinical strains of *M. tuberculosis* acted differentially in skewing the Th response. Taking this as a cue we further probed to study how these strains modulated the macrophage apoptosis and the mechanism of modulation was also studied.

Aim:

- To delineate the molecular mechanisms of apoptosis and survival strategies in infected THP-1 macrophages with different strains of *M. tuberculosis*

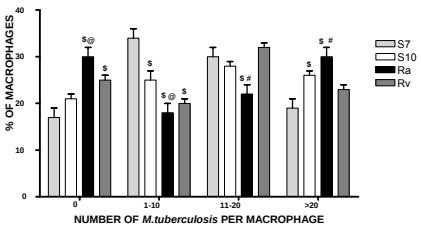
Methods:

Phorbol myristate acetate (PMA) differentiated THP-1 macrophages were infected with two clinical isolates (S7 and S10) and two laboratory strains ($H_{37}Rv$ and $H_{37}Ra$) of *M. tuberculosis* for various time points. Apoptosis was studied using annexin –V FITC labeling and survival of *M. tuberculosis* strains within the cells was detected by fluorescence microscopy. The expression of two components system *prrA–prrB* and *mprA-mprB* in *M. tuberculosis* strain was done with reverse transcriptase - PCR (RT-PCR). The cell lysates were subjected to ECL immunoblotting for various cell death and cell survival molecules.

Results:

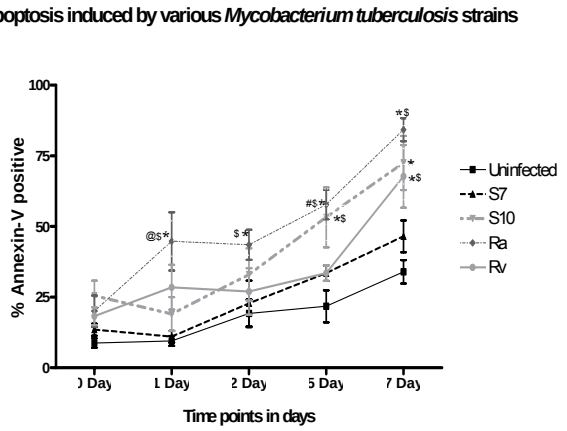
The percentage phagocytosis did not show any strain specific association with differentiated THP-1 cells. But in phagocytic index, the clinical strains showed low dose of infection in 1-10 bacilli category thereby exerted less burden on the cells (Fig 6.9 & 6.10).

Fig 6.9: Phagocytosis of various *M. tuberculosis* strains by THP-1 cells



Each bar represents the mean value of five different experiments. The statistical significance is shown as $ compared to S7, @ compared to S10, # compared to H37Rv when P<0.05.

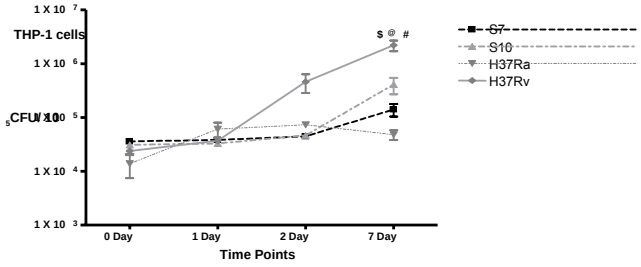
Fig. 6.10: Apoptosis induced by various *M. tuberculosis* strains



Above values are mean ± SEM obtained from four different experiments. The statistical significance is shown as * compared to uninfected, $ compared to S7, @ compared to S10, # compared to H37Rv when P<0.05.

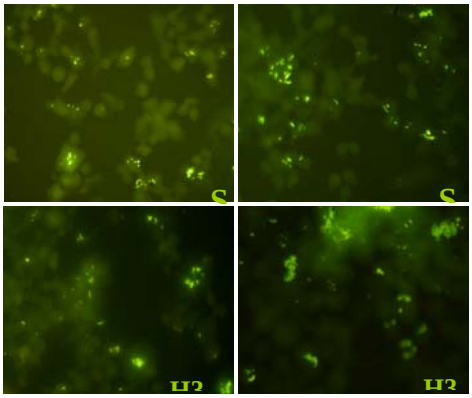
The induction of apoptosis was strain dependent (Fig 6.11 & 6.12). A delayed onset in expression of the two systems *prrA–prrB* and *mprA-mprB* in clinical strains compared to the laboratory strains was observed. At later time points of 7 and 14 days, a differential activation of these molecules in clinical isolates (S7 and S10) compared to the laboratory strains (H37Rv and Ra) was observed. Importantly, on day 7, marked upregulation of XIAP molecule was observed in S7 infected macrophages. The differential kinetics of these molecules may inhibit their initial multiplication and growth but support for longer survival in the macrophages.

Fig.6.11: Intracellular growth of various *M.tuberculosis* strains



Above values are mean ± SEM obtained from four different experiments. The statistical significance is shown as * compared to uninfected, $ compared to S7, @ compared to S10, # compared to H37Rv when P<0.05.

Fig. 6.12



Photomicrographs show THP-1 cells infected with various strains of *M. tuberculosis* (S7, S10, H37Ra and H37Rv). The infected cells were stained with auramine-KMnO4 after 3 hours of phagocytosis. The picture was taken with a fluorescence microscope (U-ND25-2; Olympus, Tokyo, Japan) at high power magnification (X400). The bacilli are bright yellow and the THP-1 cells are green.

Conclusion:

The results demonstrated differential apoptosis of *M. tuberculosis* infected THP-1 macrophages at later time points. By virtue of these pathways, virulent *M. tuberculosis* able to up-regulate the macrophage survival signal, thereby creating an environment favorable for the survival of the pathogen.

(PI: Dr. Sulochana Das; sulochanad@trcchennai.in.)

Molecular epidemiology of *Mycobacterium tuberculosis*

Background:

Mycobacterium tuberculosis has a global population structure, and there is increasing evidence that strain genetic differences have important phenotypic consequences. Early studies reported that strains from south India were less virulent in guinea pigs than strains from other regions.

Aim:

- To determine the population structure of *M. tuberculosis* in south India and identify potential genomic correlates of attenuation and virulence, using isolates from a population-based molecular epidemiologic study

Results:

To estimate the frequency of the six newly identified large sequence polymorphisms (LSPs) (Table 6.5) among the isolates with RD239 deleted, and to see whether the new LSPs were associated with different strain phenotypes,

100 isolates were chosen from the largest spoligotype clusters and screened by PCR for the six LSPs (RD768-RD773).

Table 6.5: Description of six new LSPs or genomic deletions observed in the clinical isolates from south India

Isolate	Name*	Coordinates		Primer sequence (5' …… 3')	Size† (bp)	Putative function of deleted genes and base pairs
		start	stop			
M394	RD768	70289	72023	GGG GCG GCT GTT GGA CCC GCA TAT CCT	1735	Rv0064 Transmembrane protein
M357	RD769	1957030	1959686	GAC AGC AAC CGC GAC GCC CGG AAT C	2657	Rv1730c Pencillin binding protein
						Rv1731 (*gabD*2) Succinate-semialdehyde dehydrogenase
				CCC GCC CTC GTC GTC ACC TTC ATC TGT AA		(NADP+) dependent (SSDH) *gabD*2
						Rv1732c Conserved hypothetical protein
M395	RD770	2196902	2200337	CCG GTG ACC GTC GTG GTG AGC ACC A	3436	Rv1946 (*lppG*) Possible lipoprotein
						Rv1947 Hypothetical protein
				CCA GGA CGG AGG TCA CAG TTG CGG GGT		Rv1948 Hypothetical protein
M118	RD771	89500	90450	CCG GGC GCG CGA ACA TGG ACT GC	951	Rv0081 Probable transcriptional regulatory protein
						Rv0082 Probable oxidoreductase
				GGC TCG GCG CCT CCG GGT GG		Rv0083 Probable oxidoreductase
M165	RD772	30669	34074	GCC ATC GCG GAG GCG GAA GCA GCT CT	3406	Rv0027 Conserved hypothetical protein
						Rv0028 Conserved hypothetical protein
				TTT GCC CGG CCT AGC GGT TGC CCA TC		Rv0029 Conserved hypothetical protein
						Rv0030 Conserved hypothetical protein
						Rv0031 Possible remnant of a transposase
M461	RD773	3434523	3441337	CGG CCC TGA CGG TGG CAA TCT GGA TC	6815	Rv3071-Rv3076 Conserved hypothetical
						Rv3077 Possible hydrolase
				GAG CAG GGT CGC CAG CCA GTT GCC		Rv3078 (*hab*) Probable hydroxylaminobenzene mutase
						Hab
						Rv3079c Conserved hypothetical protein
						Rv3080(*pknK*)Probable serine/threonine-protein kinase
						transcriptional regulatory protein *pknK* (protein kinase k)

bp = base pairs
* Name assigned to the specific genomic deletion
† Size of genomic deletion (bp) = (stop coordinates – start coordinates) + 1

However, RD769 and RD771 were each detected in only two of the 100 screened isolates, and RD768, RD770, RD772 and RD773 were not deleted in any of the 100 isolates. Because of the low frequency of these LSPs, we conclude that these genomic alterations are unlikely to account for the low virulence of south Indian strains in animal models.

Conclusion:

Genomic deletion analysis of 1,215 TB patient isolates from south India revealed that 85.2% belonged to the ancestral lineage of *M. tuberculosis*. We identified six new genomic regions within this lineage that were variably deleted. Our findings emphasize the need to consider global strain variation during TB product development.

(PI: Dr Sujatha Narayanan, sujathan@trcchennai.in; Funding: WHO-USAID, Collaborating Institute Stanford University)

Studies in Progress:

The role of complement in the interaction of *M. tuberculosis* with human macrophages

Background:

The complement system, an important component of innate immunity is a potent mediator of inflammation and at the same time plays a pivotal role in modulating the adaptive immune response also. The initial interaction between the complement system, the macrophage and *M. tuberculosis* is an important first step in the pathogenesis of TB and is mediated by specific macrophage receptors and ligands present on the surface of *M. tuberculosis*. Although it is known that *M. tuberculosis* replicates within the host macrophages, the mechanism by which it evades being killed by macrophages remains poorly understood. Since mycobacteria interact with the complement system initially and antimycobacterial antibodies are known to be present in endemic populations, it was considered important to investigate whether antibodies could modulate complement activation and determine the interaction of *M. tuberculosis* with the macrophage.

Aim:

- To study whether antibodies could modulate complement mediated interaction of *M. tuberculosis* with the macrophages

Methods:

Mycobacterium tuberculosis will be treated with complement (classical or alternative pathway) in the presence and absence of IgM or IgG antibodies against *M. tuberculosis.* The following will then be determined:

(i) Investigate the receptor mediated entry of *M. tuberculosis* into the macrophages.
(ii) Intracellular viability of the tubercle bacilli.
(iii) Release of free radicals and different cytokines from macrophages after interaction with *M.tuberculosis.*

Results:

Mycobacterium tuberculosis was pre treated with buffer or antibody and reacted with either the classical or the alternative pathway. This was then added to PBMCs and the expression of complement receptors and the production of cytokines are being evaluated. Of the projected 10 volunteers in whom this is to be evaluated, so far PBMC from five have been done. Preliminary results indicate that while phagocytosis is increased by the pre opsonization with complement, it is not reflected in the production of cytokines such as IL-2 or IFN-γ.

(Dr.V.D. Ramanathan, vdramanathan@trcchennai.in)

Complement activation by gene-disrupted *M. tuberculosis*

Background:

Complement system plays an important role in the opsonization and phagocytosis of mycobacteria. The alternative pathway of complement activation has the ability to recognize a variety of pathogens independent of antibody. However, both classical and alternative pathways are required for optimal phagocytosis of bacteria. Many studies have documented the complement activation potential of various mycobacterial strains and their cellular components. A number of gene-disrupted strains of *M.tuberculosis* are now available and differences in the pathogenic potential of these strains compared to the wild type strain are being delineated now. In view of the importance of the the

innate immune responses in modulating the host-parasite interactions, it is important to investigate the relationship between the complement system and genetically modified strains of *M.tuberculosis*.

Aims:

- To assess complement activation at the level of C3, C4 and factor B by the following gene-disrupted *M. tuberculosis* strains: MptpA, MptpB, VirS, DKO, DevR, complemented strains of all these except DKO and their respective wild strains, *M. tuberculosis* Erdman and H37Rv strains

- To assess the effect of the above mentioned strains of *M.tuberculosis* to modulate the expression of complement receptors on PBMCs and release of various cytokines from them

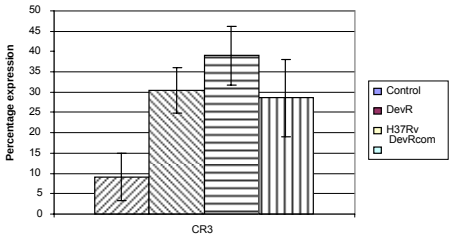
Methods:

All the above strains are grown in Middlebrook 7H9 broth for 3-4 weeks at 37^{o}C. Complement activation at the level of C3, C4 and factor B by *M.tuberculosis* will be assessed using solid phase ELISA. Complement receptor expression will be evaluated using flow cytometry and the quantitation of various cytokines using ELISA.

Results:

Whole blood cells were treated with wild type, gene-disrupted and complement strains of *M.tuberculosis.* The expression of complement receptors CR1 to CR4 using flow cytometry and the production of cytokines were assessed using bead assay by flow cytometry. Preliminary results so far from 4 individuals indicate that the mutant Dev-R induces a lesser expression of CR3 on CD14 positive cells (Figure 6.13).

Fig. 6.13: Expression of CR3 on CD14 cells



(PI : Dr.V.D. Ramanathan, vdramanathan@trcchennai.in)

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

Background:

Even though effective chemotherapy is available for treatment of TB, there are practical difficulties in ensuring the desired high cure rate, due to many factors. Immuno-prophylactic measures using vaccines is an alternative approach for control.

In the previous year's Annual Report (2005-2006), a systematic approach to test the antigens purified by two-dimensional (2-D) preparative separations, in human subjects has been described. Results of *in vitro* assays and characterization of the purified antigenic fractions are presented in this report.

Aim:

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

Methods:

The study subjects are as follows:

1. Apparently healthy household contacts (HHC) of sputum positive pulmonary TB living in the same household. Tuberculosis was ruled out in this group during the time of blood collection and hence considered "Protected".
2. Newly diagnosed adult pulmonary TB cases. They form the "susceptible" group.

The methods followed are as follows:

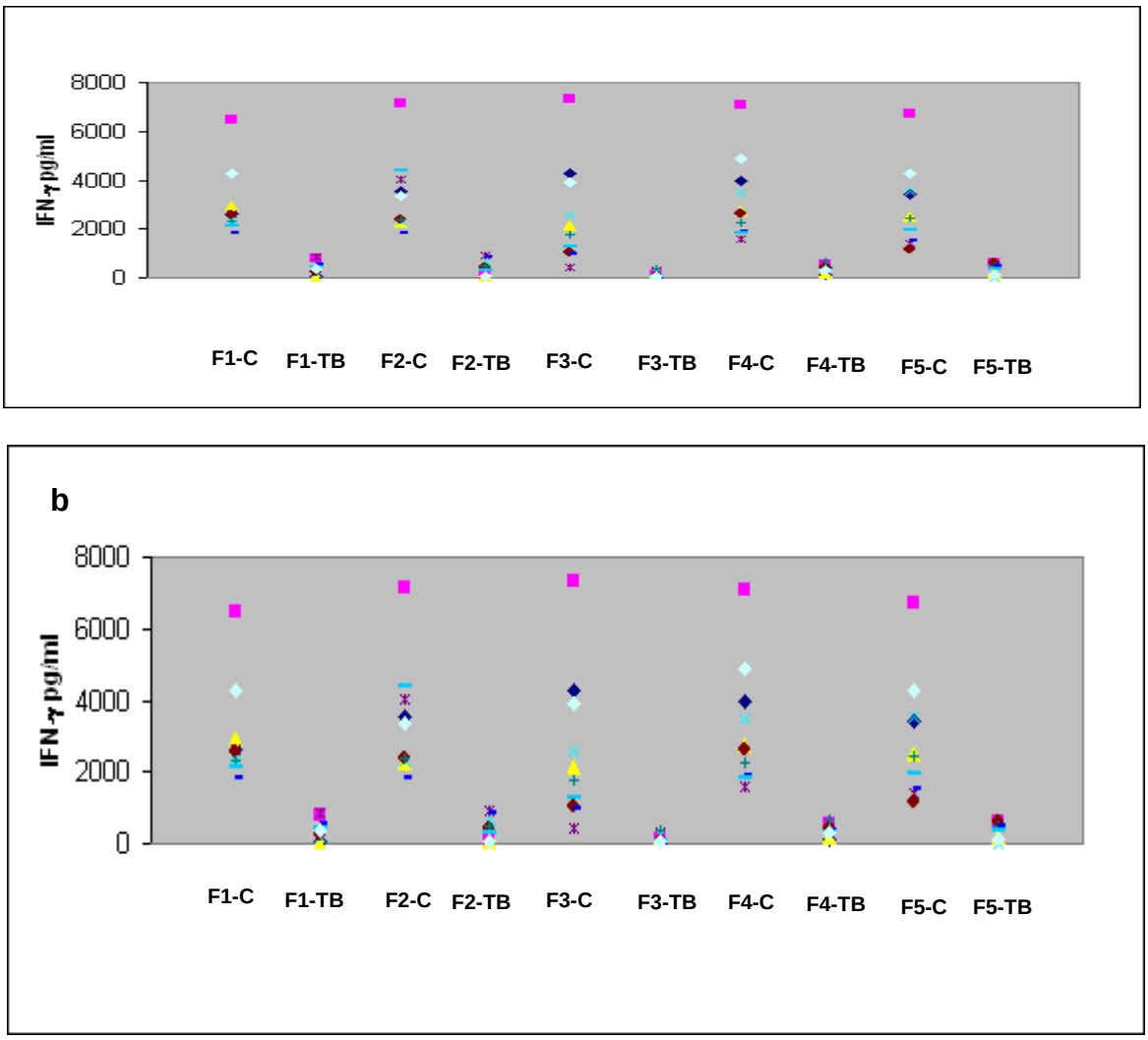
1. Two dimensional Preparatory separation of antigenic fractions
2. Proliferative response and IFN-γ response were studied using purified antigenic fractions.

Results:

Using the preparatory 2-dimensional approach, the *M. tuberculosis* secreted proteins have been separated into 600 fractions. Of these, fractions having at least 50 μg or more were selected for further testing (347 fractions). We have standardized a whole blood (1:10 dilution) assay, for proliferation and IFN-γ secretion.

Interferon-γ secretion pattern of 10 HHC and 10 TB subjects to 10 purified antigenic fractions (F1 to F10) are presented in Fig. 6.14 a & b. Out of the 10 fractions, none of them induced IFN-γ response in TB patients.

Fig. 6.14a & b: IFN-γ Response to highly recognized antigenic fractions



C-Contacts; F1-F10-antigenic fraction; TB-Tuberculosis

The rest of the fractions could be classified as those inducing response in 9 HHC and 0 TB; 8 HHC and 0 TB; 9 HHC and 1 TB; 8 HHC and 1 TB etc.

All fractions have been subjected to tandem mass spectrometry, using online multidimensional nano HPLC and electrospray ionization (ESI) – ion trap technology, with the kind help of Dr. John Belisle, Colorado State University, USA. Mass spectrum of each fraction was analyzed with Bioworks 3.3 software which uses the SEQUEST protein search algorithm and identifies the protein. Protein identity of each

fraction was further confirmed by analyzing in Scaffold Protein Prophet software, which uses SEQUEST and MASCOT protein search algorithm and results are expressed as percentage of probability of a particular protein present in the sample.
In addition, each fraction was resolved on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) followed by silver staining. Each fraction showed 1 or a maximum of 2 bands. The molecular weights and identity of the protein are being analyzed.

(PI: Dr. Alamelu Raja, alamelur@trcchennai.in)

Cytotoxic cell response in *M. tuberculosis* infection

Background:

Cytotoxic T lymphocytes are required for protective immunity against intracellular pathogens such as *M. tuberculosis*, pathogens known to escape from phagocytic vacuoles into the cytoplasm of infected host cells. Cytotoxic T cells are needed to release bacteria from the ineffective macrophages so that they can be phagocytosed by fresh, fully activated monocytes or macrophages.

Aim:

- To analyse the cytotoxic cell response in TB

Methods:

The study subjects are the two groups as described above, “susceptible” and “protected”. Response to whole *M. tuberculosis* H_{37}Rv, purified antigens such as ESAT-6 and CFP-10 and overlapping 20-mer peptides of the 2 purified antigens are being studied. Lymphoproliferation of CD4 and CD8 cells [Anti-bromodeoxyuridine (Brdu) anti BrdU labeling, flow cytometry)] and IFN-γ secretion (ELISA) to these antigens are also carried out.

Results:

Initially, cytokine response to whole *M. tuberculosis* H_{37}Rv was studied among patients and normal subjects. It was observed that there was no difference between unstimulated (control) and H_{37}Rv stimulated cells in cytokine secretion. Considering the two groups, it was observed that there was a decrease in cytokine production in

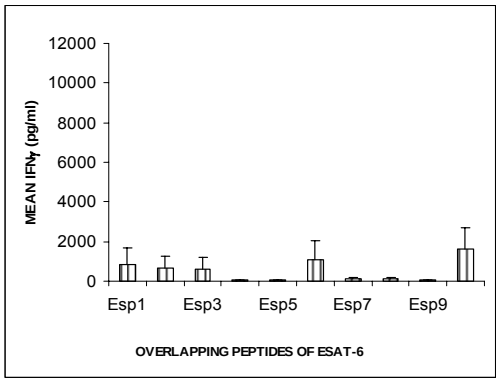
TB patients when compared to normals. The response of both CD4 and CD8 T cells was similar.

Cytolytic molecule response was also studied in these two groups to *M. tuberculosis*. There was no difference among stimulated and unstimulated cultures to various cytolytic molecule secretions. An increase in Granzyme A and B levels was observed in patients than HHC. Cytotoxic T (CD8) cells were found to produce more Granzyme A and B when compared to helper cells (CD4).

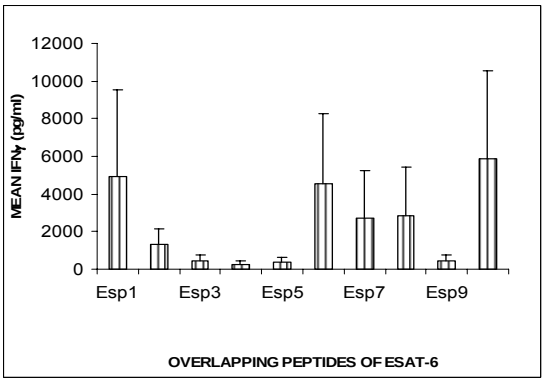
IFN-γ secretion in response to each of the overlapping 20-mer peptides of ESAT-6 and CFP-10 was measured. It was observed that ESAT-6 peptides 1, 6, 7 and 8 were immunogenic inducing IFN-γ. Peptides 7 and 8 were found to stimulate only lymphocytes from HHC and not TB (Fig. 6.15a - d).

Fig 6.15 a, b, c, d: Mean IFN-γ response among patients and contacts to overlapping peptides of ESAT-6 and CFP-10 antigen

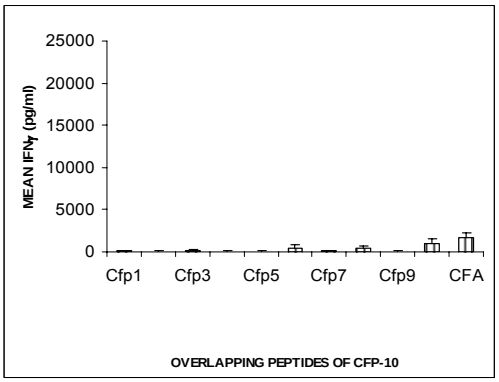
a. Patient



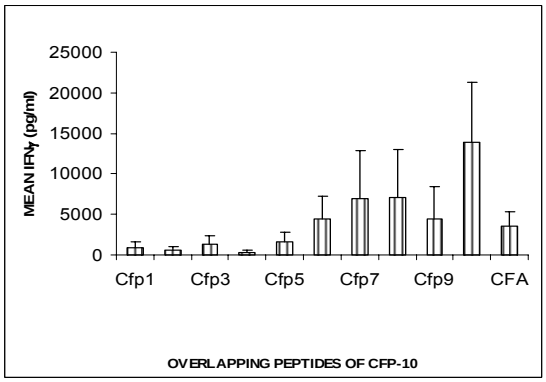
b. Contact



c. Patient



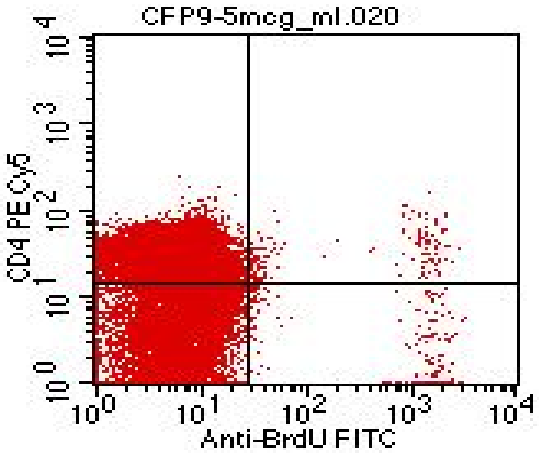
d. Contact



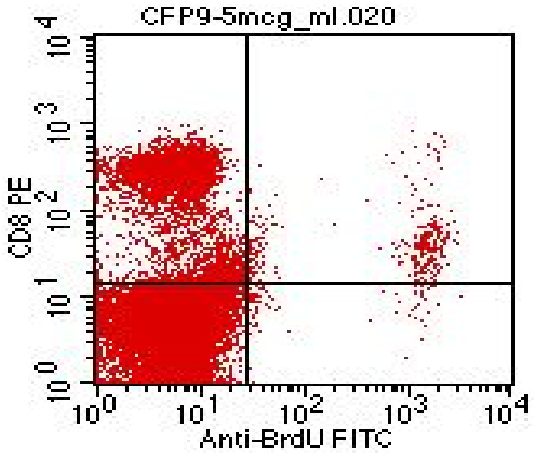
Similarly, CFP-10 peptides 7, 8 and 9 induced more response in HHC than in TB. It was observed that the peptides stimulated both CD4 and CD8 cells and proliferation to peptides [anti-Brdu labeling] was more marked in CD8 cells than CD4 T cells (Fig. 6.16a & b). .

Fig. 6.16a & b: Representative dot plot showing CFP10 - peptide 9 (5 μg/ml) responding CD4 & CD8 population by BrdU proliferation assay

a)



b)



(PI: Dr. Alamelu Raja, alamelur@trcchennai.in)

Innate immunity in HIV infection:

Background:

Natural Killer (NK) cells are innate effector cells of the immune system, which exert a prompt cytolytic activity against infected and tumor cells without prior stimulation. NK cells are active in the earliest stages of the host defense, display broad specificity and rapid activation.

Aim:

- To demonstrate NK cell mediated innate immune response in HIV-TB. Various innate immune parameters mediated through unstimulated and stimulated NK cells will be studied

Methods:

The study subjects consisted of 12 HIV seronegative TB patients and 8 NHS.

The methods to be followed are as follows:

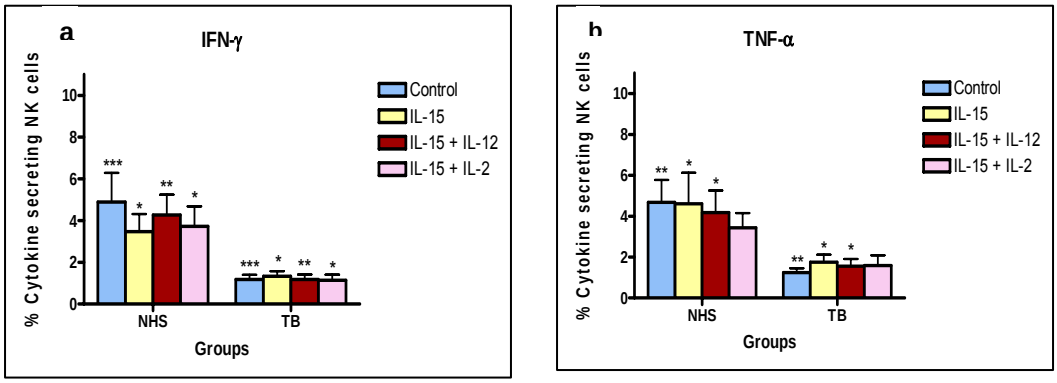
In vitro response of NK cells to *M. tuberculosis* $H_{37}Rv$ will be studied using intracellular cytokine secretion (IL-2, IL-4, IFN-γ and TNF-α). Cytotoxic molecules (Granzyme A, Granzyme B and Perforin) positive cells will be determined by flow cytometry. Response of NK cells after *in vitro* stimulation with cytokines (IL-15, IL-15+IL-12, IL-15+IL-2). Cytotoxic response of NK cells against tumor cell lines (K562) will be studied using flow cytometry.

Results:

Using *M. tuberculosis* $H_{37}Rv$ stimulation *in vitro*, both Th1 and Th2 cytokines, IL-2, TNF-α, IFN-γ and IL-4 were measured intracellularly, by flow cytometry. In both the groups, the percentage of IL-2 secreting NK cells was not altered in the presence or absence of *M. tuberculsis* $H_{37}Rv$ stimuli. Similar results were observed with other cytokines, IL-4, IFN-γ, and TNFα. The percentage of cytokine positive NK cells (>5%) was higher when compared with adaptive cells CD4 and CD8 (<2%).

It was observed that all the 4 cytokines measured *ex-vivo*, were significantly ($p<0.01$ to 0.001) reduced in NK cells of TB patients, as compared to that of NHS. Since it is known that dysregulation of cytokines play a major role in infectious diseases, the effect of *in vitro* supplementation of cytokines was studied. When the cells were stimulated *in vitro* with stimulants like IL-12+IL-15, the situation did not improve (Fig. 6.17 a & b).

Fig 6.17 a & b: Natural Killer cell response against *in vitro* stimulation with cytokines



NHS Normal healthy subjects
Pulmonary TB patients

The NK cells from both TB and NHS groups constitutively expressed cytolytic molecules such as perforin, Granzyme A and Granzyme B. The percentage of NK cells expressing cytolytic molecules did not change significantly when stimulated with H37Rv. The results were similar between TB and NHS groups. In general, cytolytic molecule positive NK cell levels were significantly greater than CD4 or CD8 cells (Table 6.6).

Table 6.6: Cytolytic response of lymphocytes against *M. tuberculosis* infection

Cytolytic molecules	NK		CD4		CD8	
	Unstimulated	H37Rv	Unstimulated	H37Rv	Unstimulated	H37Rv
Perforin	11.74	10.02	1.14	0.87	5.76	2.26
GranzymeA	37.91	29.23	5.35	3.28	26.82	18.97
GranzymeB	22.43	20.08	2.47	2.39	11.02	12.49

Values are given as Mean percentage

(PI: Dr. Alamelu Raja, 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 re-activation of latent TB infection (LTBI). 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 the role of Interferon gamma (IFN-γ), Interleukin-4 and Interleukin-4δ2 in the diagnosis of latent TB

Methods:

The study subjects are as follows:

1. Healthy household contacts (HHC), with high risk for LTBI (n = 200)
2. Healthy controls (HC), with low risk for LTBI (n = 200)
3. HIV +ve (n = 200)
4. TB +ve (n = 100)
5. HIV-TB (n = 100)

Skin test was performed and read as per standard procedures, using two TU of PPD RT23 (Statens Serum Institute, Denmark) and reading was taken 48-72 hrs post testing. Whole blood Interferon gamma releasing assay was done by using Quantiferon TB Gold kit (Cellestis, Victoria, Australia) as per the manufacturer's instructions.

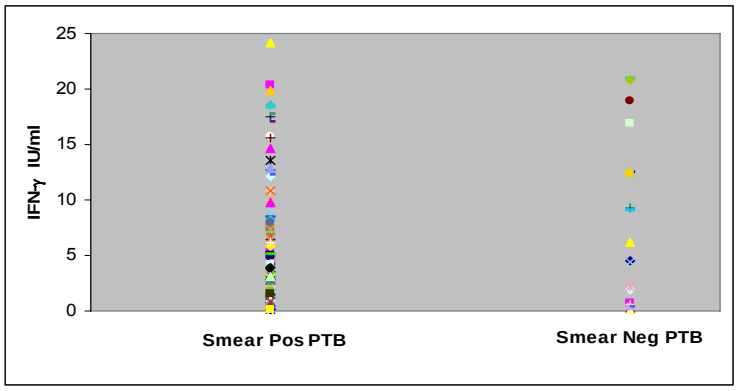
Results:

Sensitivity of interferon gamma release assay in active TB patients

Among the total number of 113 TB subjects, 81 were male and 32 were female with the median age average of 37 (Range 10-65). All the study subjects were diagnosed based on clinical, radiological and microbiological parameters.

Among the total study subjects, maximum numbers have been recruited in TB cases. The levels of IFN-γ have been measured (Fig. 6.18) 0.35 IU/ml was set as cut-off value as described by the manufacturer. IGRA showed sensitivity of 90% in both smear positive as well as negative cases. The percentage of positivity did not vary between smear positive and negative cases.

Fig.6.18: Levels of IFN-γ in Pulmonary TB patients



The horizontal line shows the kit cut-off point 0.35 IU/ml.

PI: Dr. Alamelu Raja, alamelur@trcchennai.in Funding: R03 Grant, NIH)

Human leucocyte antigen -DP (HLA-DP) and vitamin-D receptor (VDR) gene polymorphism studies in HIV and HIV-TB patients

Background:

Our recent studies revealed that HLA –A11 was associated with resistance to HIV and HIV with active TB (HIV-TB), while HLA –B40 and –DR2 were associated with susceptibility to HIV and HIV-TB. Moreover polymorphisms in VDR gene variants are shown to be associatied with differential susceptibility or resistance to TB.

Aim:

- To find out whether HLA –DP and VDR gene variants are associated with susceptibility or resistance to HIV and HIV-TB

Methods:

The study subjects include 151 HIV positive TB negative patients (HIV+TB-), 117 HIV positive TB positive patients (HIV+TB+), 150 HIV negative TB positive patients (HIV-TB+) and 150 healthy controls. VDR-Taq1 polymorphism was studied using PCR with sequence specific primers followed by digestion with Taq1 restriction enzyme using RFLP method.

Results:

An increased frequency of Tt genotype was observed among HIV patients without TB as compared to controls (p=0.02) while a decreased frequency of 'tt' genotype was seen in HIV patients without TB compared to HIV patients with TB (p=0.02) (Table 6.7). Studies on other polymorphisms in VDR gene (CDX2, A1012G, Fok1, Bsm1 and Apa1) are in progress. Studies on HLA –DP typing are in progress.

Table 6.7: Percent genotype frequencies (%GF) of VDR Taq1 polymorphism among healthy controls, HIV patients without and with TB

VDR Taq 1 genotypes	Healthy controls (n=132) %GF	HIV+ TB- (n=148) %GF	HIV+ TB+ (n=114) %GF
TT	47 (62)	36.5 (54)	36.8 (42)
Tt	40.9 (54)	54.05 (80)	43.7 (50)
tt	12 (16)	9.5 (14)	19.3 (22)

Numbers in the parentheses represent individual positive for that genotype
n= number of subjects studied

(PI: Dr.P.Selvaraj; selvarajp@trcchennai.in)

Role of variant genotypes of vitamin-D receptor gene on plasma vitamin D_3, vitamin D receptor expression and intracellular cytokine positive cells in pulmonary tuberculosis

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 gene 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

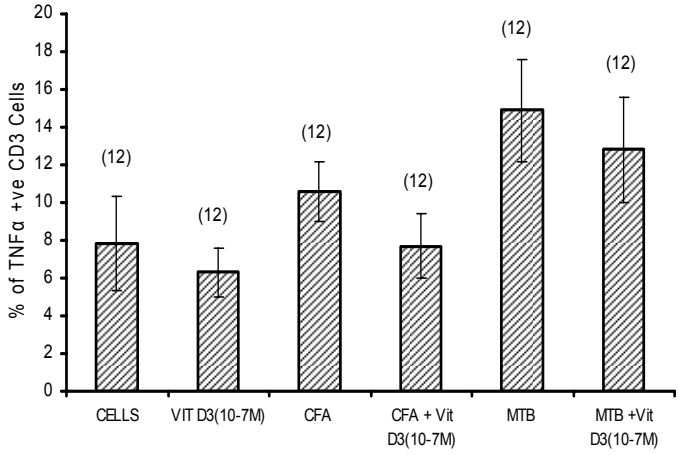
Methods:

Study subjects include 70 pulmonary TB patients and 70 normal healthy subjects. Enumeration of TNF-α and IFNγ positive cells will be done in PBMC cultures by flow cytometry at various time points. Portion of the PBMCs will be used for VDR protein assay and DNA extraction for genotyping of VDR. Serum vitamin D_3 will be estimated using commercial ELISA kits.

Results:

The effect of vitamin D_3 on TNF-α cytokine expression at the intracellular level has been studied in 12 normal healthy subjects so far. A trend towards a suppressive effect of vitamin D_3 was observed on TNF-α expression in CD3 positive cells stimulated with live *M.tuberculosis* and culture filtrate antigen (Fig 6.19). Vitamin D receptor protein assay has been standardized. The study is in progress.

Fig.6.19: Effect of vitamin D_3 on intracellular TNF-α positive CD3 cells in NHS



(PI: Dr.P.Selvaraj; selvarajp@trcchennai.in)

Cytokine gene polymorphisms and cytokine levels in pulmonary tuberculosis

Background:

Th1 and Th2 cytokines play an important role in the immune response against TB and alteration in their levels contribute to the out come 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 whether variant genotypes of cytokine genes and cytokine levels are associated with susceptibility or resistance to pulmonary TB

Methods:

The study subjects include 150 pulmonary TB patients and 150 normal healthy subjects. Genotyping will be done by PCR- allele specific primer (PCR-ASP), PCR- allele specific oligo nucleotide probe (PCR-ASOP) and PCR-RFLP method.

Results:

Among the various cytokine gene polymorphisms studied, none of the variant genotypes of IL-8 gene (-251 T→A) was found to be associated with susceptibility to TB. TT genotype was associated with higher IL-8 production (p=0.05). There was a lack of association of Interferon γ +874A polymorphism with TB. Significantly increased frequency of IL-4 CT genotype of (IL-4 -590 C→ T polymorphism) in patients (p<0.05) and CC genotype in NHS (p<0.01) was observed. No significant difference among the variant genotypes of IL-12p40 and IL-10 gene polymorphisms were noted (Table 6.8). IL-12p40 and IL-10 gene polymorphisms will be done in more number of samples to confirm the results. Studies on IL-18 gene polymorphisms are in progress.

Table 6.8: Variant genotype frequencies of IL-12p40 and IL-10 gene in normal healthy subjects and pulmonary TB patients

IL-12p40 and IL-10 Polymorphisms	Genotypes	Genotype frequency (%)	
		NHS n=70	PTB n=70
IL-12 p40 3'UTR (C/A)	CC	21.42(15)	14.28(10)
	CA	51.42(36)	57.14(40)
	AA	27.14(19)	28.57(20)
IL-10 (-1082 A/G)	AA	51.42(36)	48.57(34)
	AG	40.00(28)	47.14(33)
	GG	8.57(6)	4.28(3)

n= number of subjects. Numbers in parentheses represent subjects positive for each genotype.

(PI: Dr.P.Selvaraj; selvarajp@trcchennai.in)

Evaluation of cellular immune response to HIV-1 infection with special reference to HIV gag epitope mapping among south Indians

Background:

HIV-specific cytotoxic T lymphocytes (CTLs) play a major role in the control of viral replication. However, a complete understanding of the specific epitope targeted by CTLs is essential to assess this antiviral response.

Aim:

- To identify the immunodominant epitopes recognized in HIV-1 infected south Indian population using gag overlapping peptides

Methods:

A total of 30 HIV infected patients at various stages of diseases have been screened so far. Twenty ml venous blood was obtained from each patient after obtaining written consent. PBMCs were separated by density gradient centrifugation and enzyme linked immunospot (ELISpot) assay was performed using HIV-1 gag peptides obtained from the NIH AIDS repository (overlapping peptides were pooled in such a way that each peptide was found in two peptide pools). The plates were processed further on the next day and subsequently the ELISpots were analyzed on a Carl Zeiss Axioplan ELISpot reader. Peptide matrix technique was used to zero down on the peptide responsible for spot formation. HLA typing of the study subjects will be performed to determine HLA restriction of CTL response.

Results:

We have identified 21-peptide sequences with possible putative epitopes (sequences not shown) (Fig 6.20). The epitopes will be identified after HLA typing and using the Epitope Location Finder (ELF) software.

Fig. 6.20: Representative Elispot assay using HIV-1 gag overlapping peptide pools

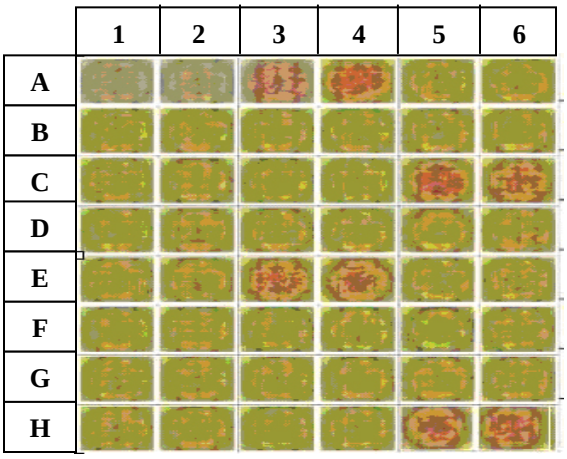


Figure Legend :

Wells A1, A2 – Negative Control
A3, A4 – Positive Control
C5, C6 – Pool 7 – Tested positiove
E3, E4 – Pool A – Tested positive
H5, H6 – Pool K – Tested positive

Elispot plate showing wells with spots (positive)

PI: Dr.Soumya Swaminathan; soumyas@trcchennai.in)

Effect of anti tuberculosis treatment and antiretroviral therapy on intracellular cytokine production in CD8 T cell subsets of HIV+TB+ patients

Background:

In any infection, naïve CD8 T cells are primed and consequently proliferate and differentiate into effector and memory cells. Peripheral effector CD8 T cells show direct effector function such as cytotoxic activity and cytokine production in response to their targets, whereas memory CD8 T cells do not have direct cytotoxic activity without prior activation but can proliferate and secrete a large amount of cytokines in response to antigenic stimulation. There is a gap of knowledge as to what happens to the CD8 T cell maturation pathway during ATT and ART in HIV+TB+ patients.

Aim:

- To analyze the expression of intra cellular cytokines (IFN-γ IL-2, TNF-α) in phenotypic subsets (naïve, memory, memory effector and effector) of CD8 T cells in patients with HIV and active TB and to study the effect of ATT and ART on cytokine production in different CD8 T cell subpopulations

Methods:

Peripheral blood samples will be obtained from ATT and ART naïve HIV+TB+ patients at baseline (time of entry into the study), 2 months after initiation of ATT and subsequently 1, 4 and 6 months after initiation of ART. Whole blood cells will be stimulated with PMA + ionomycin, purified protein derivative (PPD) and HIV-1 P24 antigen with/without brefeldin A. Cells will subsequently be stained with fluorescence-tagged monoclonal antibodies directed against cell surface markers viz CD8, CD45RA and CCR7 along with internal markers for IFN-γ, IL-2 and TNF-α, Flow cytometric analysis will be performed using a BD FACS calibur. The study is in progress.

(PI: Dr.Soumya Swaminathan; soumyas@trcchennai.in)

Role of chemokines in tuberculous immunity: Role of CXC-type of chemokines and its receptors in tuberculosis

Background:

Pulmonary TB is characterized by granulomatous inflammation with an extensive infiltration of mononuclear cells. The immune response against *M. tuberculosis* is typically T helper 1 (Th1) dominated. It is well established that CD4 T-effector functions. Interferon gamma production, cytolysis of infected monocytes and macrophages etc., are important for bacterial clearance. The recruitment of leukocytes occurs via the regulating chemokines (CXC or CC-type) and its cognate receptors expression on the immune cells is one of the most fundamental host defences for effective containment of the disease. Both CXC – and CC-type of chemokines is recognized as important mediators in a variety of inflammatory states especially during TB. Moreover, the recent studies highlight the importance of CXC type of chemokine in driving the T cells to the site of infection, particularly during TB.

Aim:

- To evaluate and compare expression of selected α-type (IP-10, MIG and IL-8) of chemokines and their receptors (CXCR2, CXCR3) on the immune cells (T cells, B cells, NK cells, monocytes and neutrophils) in the peripheral blood of healthy normal individuals and PTB patients

Methods:

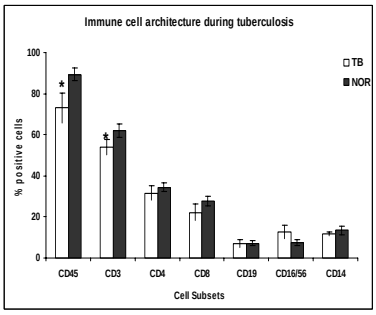
Blood was collected from the normal healthy individuals and PTB patients. The profiling of the immune cells was done by using BD Simultest kit. The cells were dual labeled for various chemokine receptors and analyzed by flow cytometry. Chemokines were assayed in plasma by the cytometric bead array (CBA).

Results:

The cell profile analysis showed a significant decrease only in CD3 T cells (Fig-6.21a) in PTB patients with no significant change in other cell types when compared to normals. The CXCR2 expression was significantly increased on T, NK cells and neutrophils but decreased on monocytes in PTB (Fig.6.21b). The CXCR3 expression was highly significant in PTB patients on CD4 T cells and not

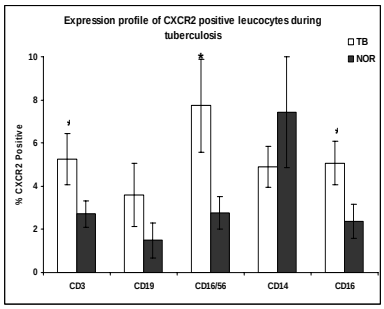
on B cells (fig.6.21c). The IP-10, MIG and IL-8 levels were significantly higher in PTB patients (fig.6.21d).

Fig. 6.21a



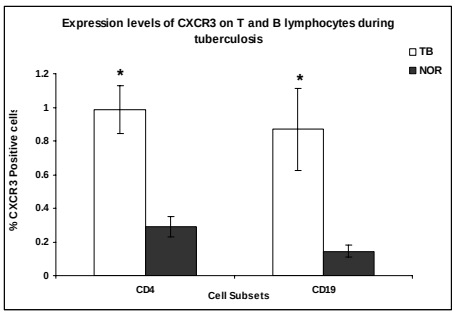
*(p<0.05 denotes the statistical significance between normal and PTB subjects.

Fig. 6.21b



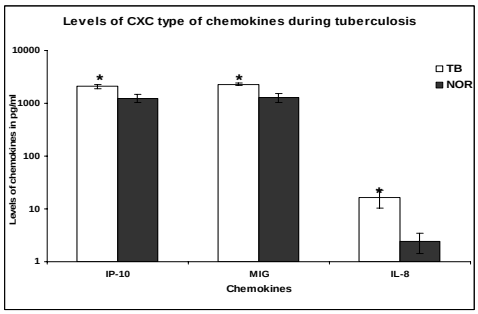
* (p<0.05) denotes the statistical significance between normal and PTB subjects.

Fig. 6.21c



* (p<0.05) denotes the statistical significance between normal and PTB subjects.

Fig. 6.21d:



* (p<0.05) denotes the statistical significance between normal and PTB subjects.

Conclusion:

Our results demonstrate that the CXC type (IP-10, MIG and IL-8) of chemokines and their receptors (CXCR2 and CXCR3) play an essential role in driving the specific immune effector cells to the site of infection to confine the bacilli within the granuloma and combat the disease progression.

(PI: Dr.Sulochana Das; sulochanad@trcchennai.in)

Role of dendtritic cells in mycobacterial immunity: Modulated function of monocyte derived dendritic cells in pulmonary tuberculosis

Background:

Tuberculosis remains one of the challenging diseases to mankind. Despite continuous surveillance and treatment, it is causing a mortality rate of 2 million per year. This failure in prevention of the disease is due to poor understanding of the interaction between host immune and mycobacterial virulence factors. In the immune system, dendritic cells (DC) play a dual role in the immune response, participating in its induction, and the maintenance of immune tolerance. They are likely to play an important and unique role in the generation of protective immunity to mycobacteria. Dendritic cells are derived from different cellular origin of which monocytes derived DCs (MODC) contribute considerably to the circulating DCs. These DCs when encounter an antigen lead to activation and migration to secondary lymphoid organ where they trigger a specific Tcell response.

Aim:

- To perform phenotypic and functional analysis of MODC generated *in vitro* in the presence of GM-CSF and IL-4 from PTB patients and to evaluate their maturation status with lipopolysaccharide (LPS) stimuli.

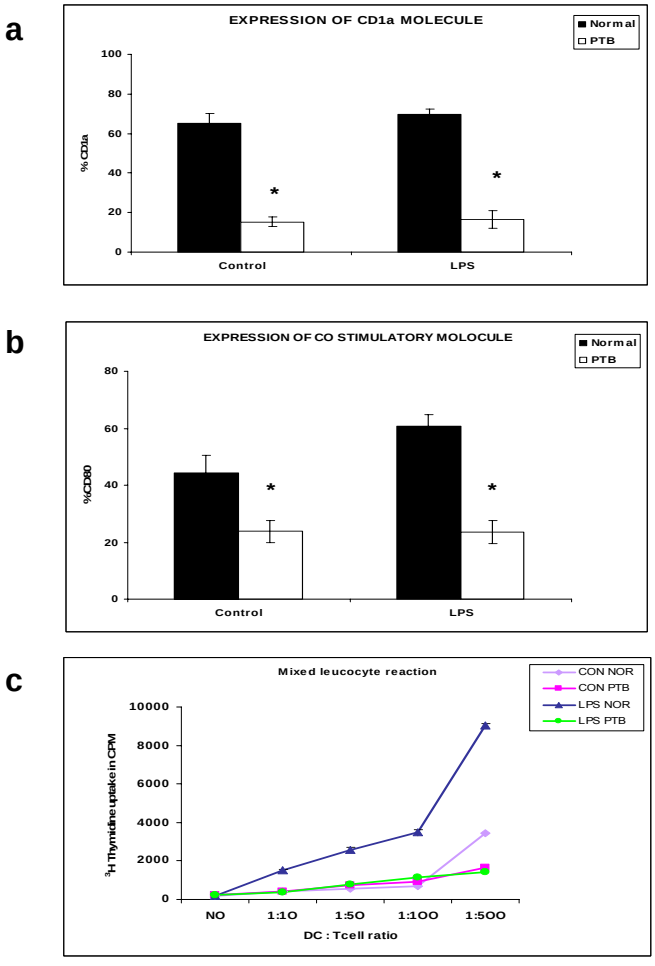
Methods:

Peripheral blood mononuclear cells were isolated by density gradient centrifugation from the blood of recruited healthy individuals and PTB patients. Monocytes were purified using anti-CD14 conjugated magnetic beads. MODC were generated by culturing $CD14^+$ cells with GM-CSF and IL-4 for 6 days. On day 7, the MODC were either stimulated with or without LPS treatment. After 24 hours, DCs were harvested and analyzed using FACS for various phenotypic markers. ELISA for cytokines was performed in the culture supernatants. The infected MODC were subjected to allogenic mixed leucocytes reaction to determine the activation of T cells by MODC.

Results:

The expression of CD1a was markedly lesser in MODC of PTB patients compared to normal individuals but the expression of CD14 was almost absent in the MODC of both subjects (Fig.6.22a). These cells also showed lower levels of HLA class II and CD80 (B7.1) in both control and LPS stimulated group with respect to patient's MODC (Fig.6.22b). Surprisingly the levels of CD86 were up-regulated in differentiated-DCs of PTB patients. Interestingly, MODC derived from PTB subjects revealed an impaired antigen-presentation function as assessed by the reduced capability to induce proliferation of T lymphocytes (Fig.6.22c). These results were then correlated with pro-inflammatory and Th1 cytokines.

Fig 6.22a, b, c



The statistical significance is shown as * ($p<0.05$) compared to normal and PTB.

Conclusion:

Our results support the notion that there is an interference with monocyte differentiation into fully competent DC in PTB patinets. This interference may be due to the exposure of the precursor cells with *M. tuberculosis* antigen. Hence may be an evasion mechanism of *M. tuberculosis* that could contribute to its intracellular persistence avoiding immune recognition.

(PI: Dr.Sulochana Das; sulochanad@trcchennai.in)

Activation of mitogen activated protein kinases by *M. tuberculosis* strains in monocytes/macrophages

Background:

During the early stages of *M. tuberculosis* infection, the control of the intracellular bacterial survival and proliferation is dependent on the macrophage innate resistance. Both mouse and human studies have provided ample evidence that even in the face of an adequate immune response, mycobacteria like *M. tuberculosis* and *M. avium* are able to persist inside macrophages. Of interest, several strains and distinct morphotypes of *M.avium* differ with respect to virulence and persistence in an *in vivo* infection model. Apart from the other vital evasion mechanisms mentioned above, one potential mechanism by which virulent mycobacterial strains, as opposed to avirulent strains, may achieve a state of long-term persistence is the modulation of signaling cascades leading to macrophage activation. Among the diverse signaling cascades that are involved in triggering cellular responses to pathogenic organisms, one essential branch of cell signaling in eukaryotic organisms is the ubiquitously expressed family of mitogen activated protein kinases (MAPK). The MAPKs are serine/threonine protein kinases that respond to a range of external and intracellular stimuli and coordinate signals for diverse cellular functions from cellular growth and proliferation to apoptosis and extracellular-signal regulated kinase (ERK) which has p44 (ERK1) and p42 (ERK2) isoforms. The JNK and p38 are primarily induced in response to cellular stress, osmolarity, heat shock, UV irradiation and also in response to inflammatory cytokines. ERK is mainly activated by growth factors and phorbol esters.

Aim:

- To study the host signal transduction pathways induced in response to *M. tuberculosis* infection

Methods:

The present study mainly focuses on finding out the differential induction of the MAPK in the primary human monocytes and THP-1 cell line (human monocytic cell line), upon challenge with *M. tuberculosis* $H_{37}Rv$, $H_{37}Ra$, drug resistant Beijing strain (DRB) and low virulent clinical strains isolated in south India. Results of this study will shed light into the mechanisms of colonization of mycobacteria in monocytes/macrophages and also the specific signaling pathway which it triggers in order to combat the host immune responses.

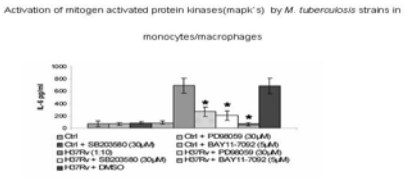
Results:

THP-1 cells were infected with *M. tuberculosis* $H_{37}Rv$, $H_{37}Ra$, DRB and two low virulent clinical strains and the activation of ERK1/2, p38 mapkinases were blotted at 45 min time point.

To decipher the pathway that is utilized by *M. tuberculosis* H37Rv to induce IL-6 cytokine secretion in primary human monocytes, inhibition studies were carried out.

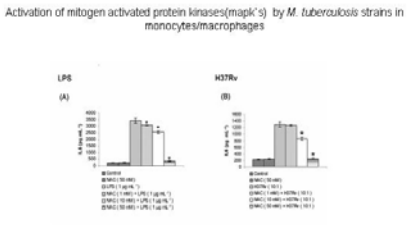
Human primary monocytes were preincubated with pathway inhibitors - 30 μM PD98059 (MEK1 inhibitor), or SB203580 (p38 MAPK inhibitor), or 5 μM Bay 11-7082 (NF-κB inhibitor) for 60 min before incubation with *M. tuberculosis* $H_{37}Rv$ for 24 hr, and cellfree supernatants were assayed for IL-6 secretion. IL-6 production was significantly reduced by all the three inhibitors used — 30 μM PD98059, 30 μM SB203580 and 5 μM Bay 11-7082 inhibited 61.6, 70.39 and 90% respectively. These results show that ERK1/2, p38 MAPK and NF-κB are involved in the signaling of IL-6 production during mycobacterial infection of human primary monocytes (Fig.6.23, 6.24, 6.25)

Fig 6.23 :



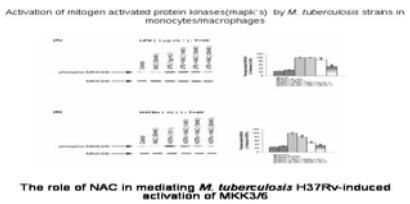
ERK1/2, p38 MAPK and NF-κB pathways are critical for *M. tuberculosis* H37Rv-induced IL-6 formation in human primary monocytes

Fig 6.24 :



NAC down-regulates IL-6 formation in THP-1 cells incubated with *M. tuberculosis* H37Rv or LPS

Fig. 6.25 :



The role of NAC in mediating *M. tuberculosis* H37Rv-induced activation of MKK3/6

Conclusion:

The activation of MAPKs by different strains was different. It has also been shown that ERK1/2, p38 MAPK and NF-κB are involved in the signaling of IL-6 production during mycobacterial infection of human primary monocytes.

(PI : Dr. Sujatha Narayanan; sujathan@trcchennai.in)

Recombinant expression, purification and characterization of low molecular weight penicillin binding protein DacB2 of *Mycobacterium tuberculosis*

Background:

The biosynthesis of bacterial cell wall peptidoglycan involves several cytoplasmic and membrane bound enzymes, which include the Penicillin binding proteins (PBPs). The PBPs are the target enzymes for beta-lactam antibiotics. They are members of the penicilloyl serine transferase family of enzymes, which carry a serine residue at the active site and are characterized by structural motifs unique to each category. PBPs play an essential role in cell division and morphology. The membrane bound PBPs can be broadly classified into the high-molecular weight (HMW) and low molecular weight (LMW) PBPs. The HMW PBPs appear to be important in cell elongation, septation, or shape determinants. The LMW PBPs usually catalyze acyl transfer reactions. The DacB2 protein is the low molecular weight PBP and the function of this is unknown.

Aim:

- To purify and characterize the DacB2 protein of *M. tuberculosis*

Results:

The dacB2 gene of *M. tuberculosis* was PCR amplified (870bp) and it was cloned into PET 43.1 vector. The clones were confirmed by restriction enzyme digestion. One clone (BA6) was chosen for induction studies. The recombinant protein consists of the *nus* and other tags (67kDa) and the Dacb2 protein (33kDa). The expressed protein was confirmed by Western blot. The recombinant protein was purified using Ni-NTA agarose columns specific for the 6XHis tag present in the expressed protein. The pencillinase activity of expressed recombinant DacB2 was carried out by with ^{14}C benzyl penicillin. The assay was performed with different concentrations of protein and ^{14}C benzyl penicillin. The concentration of ^{14}C benzyl penicillin was standardized to 1 µCi per reaction. At this concentration the dialyzed samples gave moderate signals after 10 days exposure.

Conclusion:

The result shows that expressed recombinant DacB2 protein of *M. tuberculosis* is functionally active. Future studies involves site directed mutagenesis in serine residue of DacB2 and protein protein interaction, speculated to regulate the activity of β-lactamases and related proteins like penicillin binding proteins thus indirectly controlling cell viability, cell shape or morphology.

(PI : Dr. Sujatha Narayanan; sujathan@trcchennai.in)

Recombinant BCG vaccine using epitope delivery system for *Mycobacterium tuberculosis*

Background:

One of the highest priorities of TB research is a vaccine more potent in humans than the current vaccine, BCG. Despite widespread use of BCG, TB continues to ravage humanity. Each year, *M. tuberculosis*, the primary causative agent of TB, causes approximately 8 million new cases of TB and 2 million deaths, making it the world's most lethal infectious agent. Adding to the problem, strains of *M. tuberculosis* resistant to the major antibiotics used to treat TB are emerging rapidly worldwide.

Viable carrier systems offer the greatest potential for innovative approaches to develop polyvalent vaccines. Efficient protection against infectious agents often requires the action of both humoral and cellular immune mechanisms. Therefore, an ideal polyvalent antigen delivery system should be capable of stimulating all desired effector cell populations of the immune system. Live replicating bacteria and viruses that stimulate complex immune responses have been rendered avirulent and endowed with the ability to express foreign proteins derived from pathogenic microorganisms

In the previous study we expressed HIV-1 PND epitope in BCG in the carrier antigen background. Two versions of the Cpn10-PND chimeric antigens were constructed and expressed in *M. smegmatis:* 1. the replacement chimera where the PND epitope replaces the Cpn10 loop and 2. the insertion chimera where the PND epitope is inserted into the Cpn10 loop. The expression profiling was analysed in *E. coli*, *M. smegmatis* and BCG which showed mycobacteria specific

expression. The construct was then electroporated into BCG Pasteur. Sub-cellular localization showed that the chimeric antigen was present in the culture supernatant and the cell wall. The immunogenicity of the recombinant BCG (y BCG) was evaluated in a murine model. Vaccination with rBCG expressing the PND epitope induced both cellular and humoral immune responses as measured by lymphocyte proliferation, delayed-type hypersensitivity (DTH) reaction, cytokine secretion, generation of memory T- cells and antibody production.

Aim:

- To express *M. tuberculosis* specific epitopes in BCG using the epitope delivery system
- To evaluate the protective efficacy and immunogenicity of the rBCG constructs in a suitable model of infection.

Results:

Understanding the nature of the host–pathogen interactions at different stages of TB is central to new strategies for developing chemotherapies and vaccines. The antigens were selected based on their role at different stages of *M. tuberculosis* pathogenesis. The antigens included RD1 antigens, mammalian cell entry protein, markers for latency (icl, hsp X) and genes upregulated under *in vivo* conditions in a mouse model of infection. The putative promiscuous and high affinity binding to Class I/II major histocompatibility complex (MHC) molecules were selected based on literature search and by bioinformatics epitope prediction, BIMAS (Bioinformatics And Molecular Analysis Section) (http://thr.cit.nih.gov/molbio/hla_bind/} which ranks potential 8-mer, 9-mer, or 10-mer peptides based on a predicted half-time of dissociation to HLA class I molecules. The analysis is based on coefficient tables deduced from the published literature by Dr. Kenneth Parker, Applied Biosystems. In this algorithm, binding of the epitope to 33 different human MHC class I molecule and 9 different H2 alleles of mouse is estimated.

The epitopes for inclusion into the epitope delivery system were selected based on the following criteria:

- High affinity binding to MHC allele

- Promiscuity of the epitope
- Binding of the epitope to the frequently occurring MHC allele
- Function of the protein
- If exported or intracellular or transmembrane
- Overall percentage of the protein in whole cell lysate

(PI : Dr. Sujatha Narayanan; : sujathan@trcchennai.in)

Molecular Characterization of *cis* and *trans* acting elements of Acetamidase Operon of *Mycobacterium smegmatis*

Background:

Studying mycobacterial gene regulation at the promoter level is an important goal in the mycobacterial genetics. It is essential to understand the gene expression machineries of mycobacteria related to transcription mechanisms, since many of the mycobacterial genes are not successfully expressed by the well established *E. coli* promoters. Molecular characterization of the many mycobacterial genes and assessment of the drug susceptibility and sensitivity for clinical isolates were hampered by the lack of good homologous mycobacterial expression vectors. 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 is expressed in basal level in non-induced conditions and 100 fold induced in the presence of an inducer like acetamide. This enzyme is a part of an operon, the acetamidase operon of *M. smegmatis* which has other four predicted open reading frames (ORFs), which could be involved in the expression and regulation of this operon.

Aim:

- To characterize the promoter of this operon and to construct a stable and suitable expression vector for mycobacteria.

Results:

The predicted four ORFs (Orf C, 1, 2 and 3) upstream to the acetamidase enzyme were cloned and two of which were successfully expressed in *E .coli* expression system and purified. Orf 1 was speculated to be a MarR family of

repressor and it is predicted to have a Helix-turn-helix motif and Orf 2 is predicted to have a zinc finger motif. These two proteins were assessed of their ability to bind with the *cis* acting elements of the operon by Electro Mobility Shift Assays (EMSA) (Fig. 6.26, 6.27, 6.28).

Fig. 6.26: Expression kinetics of pETOrf1 in *E. coli*BL21 (DE3)

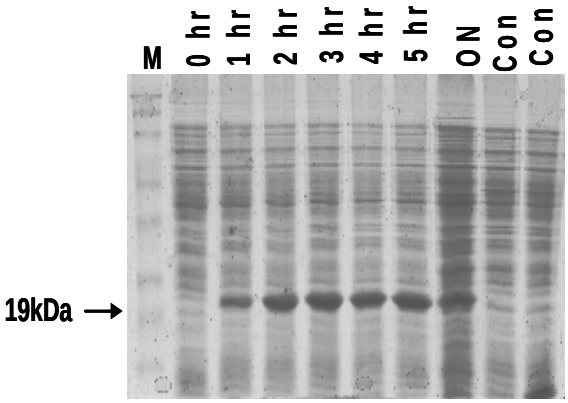


Fig. 6.27: Expression profile of pETOrf2 in *E. coli*BL21 (DE3)

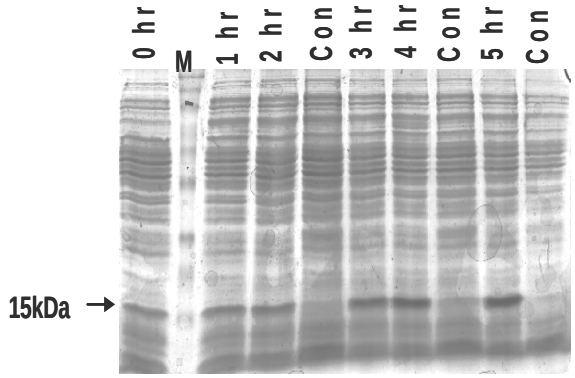
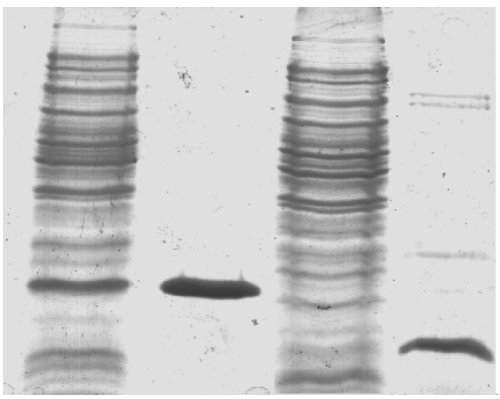


Fig. 6.28: Immobilized Metal Affinity column Chromatographic Purification of AmiA and AmiD



Lane 1 - Crude lysate of pETOrf 1/ BL21 (DE3), lane 2 – AmiA Purified protein, lane 3 - Crude lysate of pETOrf 2/ BL21 (DE3), lane 4 – AmiD Pruified protein

Conclusion:

One of the expressed proteins binds to the various upstream sequence of the acetamidase operon. Foot printing analysis will be carried out to find the exact DNA binding site for these proteins on the operon and other protein science tools like Circular dichroism spectroscopy would be used to characterize these proteins.

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In vitro studies on serine / threonine protein kinase PknE from *Mycobacterium tuberculosis* $H_{37}Rv$

Background:

Protein kinases play a cardinal role in signal-transduction pathways executing different cellular activities such as adaptation and differentiation in prokaryotes and eukaryotes. Signal transduction in prokaryotes usually use histidine kinases whereas in eukaryotes it is mediated by Ser/Thr or Tyr kinases. Interestingly, analysis of genome sequences revealed the presence of putative genes encoding eukaryotic-type Ser/Thr kinases (STPKs) in many bacterial species and in *M. tuberculosis* genome there are 11 STPKs among which PknE is of our interest. In our previous report we have shown that the gene disrupted mutant has less intracellular survival and shown resistance to various compounds especially to nitric oxide donors.

Aims:

- To study the role of PknE gene in cellular processes – mechanisms of cytotoxicity (apoptosis and necrosis) and proliferation
- To estimate the cytokine secretion, nitrite and glutathione levels after macrophage infection

Methods:

THP-1 cells were differentiated in to macrophages by PMA and subsequently infected with *M.tuberculosis* H_{37}Rv [wildtype], ▲E, C▲E (complemented PknE) for 1st, 3rd, 5th, 7th days. The infected culture supernatants were used for cytokine profiling namely TNF-α, IL-6, IL-4, IFN - γ, IL-10, IL-12p70 by ELISA. The cells were used for AnnexinV – propridium iodide (PI) staining and tunnel assay. To assess necrosis, lactate dehydrogenase assay was done and the nitrite levels were estimated by Griess assay.

Results:

The levels of IL-4, IFN -r, IL-10, IL-12p70 were not significantly different between the wild type and mutant strain of *M.tuberculosis*. The levels of TNF-α, IL-6, levels were significantly reduced in the mutant (Fig-6.29). TNF-α, IL-6, are important pro inflammatory cytokines where TNF is involved in inducing apoptosis and IL-6 in provoking IFN–r response. AnnexinV –PI staining detected early apoptotic events in infected cells. Tunnel assay was used to validate late apoptotic events [Fig-6.30]. Both showed increasing apoptotic events with reference to PknE. LDH assay showed that mutants released low levels of LDH confirming the apoptotic assays done. Proliferation marker staining showed decreased proliferation indicating that apoptosis is occurring. Griess assay showed no difference in the nitrite levels. Glutathione levels were also reduced in the mutant suggesting that the infected cells were dying.

Fig.6.29 :

Fig-1 ***In vitro*** **studies on serine / threonine protein kinase PknE from** ***Mycobacterium tuberculosis*** **H37RV**

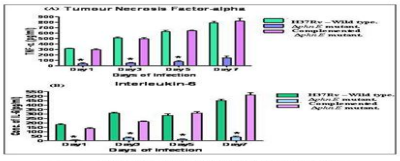
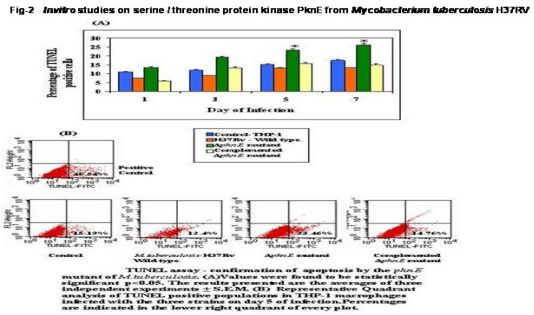


Fig.6.30 :



Conclusion:

The serine threonine kinase Pkn E has a role in apoptosis, nitric oxide metabolism and innate immune response of *M.tuberculosis* infected THP1 macrophage cell line .

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7. STATISTICAL RESEARCH

Ongoing Study:

Classification using Simplified Fuzzy Adaptive Resonance Theory Map

Simplified Fuzzy Adaptive Resonance Theory Map (SFAM) is a family of neural networks that performs incremental supervised learning of recognition categories and multidimensional maps of both binary and analog patterns. SFAM is fast, interactive, incremental and stable and it has been applied for prediction in many areas. Medical diagnoses present many challenges in classifying patients based on symptoms. One of the major problems in medical diagnosis is the subjectivity involved in classification.

Artificial Neural networks (ANN) are preferred for solving these problems because of their parallel processing capabilities as well as decision making abilities. ANNs have been applied for various medical classification tasks such as predicting prognosis and survival rates. ANN is used as an information analysis tool, which provides valuable aid for pattern classification. The Neuro-Fuzzy systems have also been applied for the solution of various pattern classification problems.

Adaptive Resonance Theory (ART) neural networks were originally proposed by researchers for pattern classification. ART1 was developed allowing unsupervised classification of binary inputs and ART2 was developed allowing unsupervised classification of analog inputs. This network uses normalized and complemented inputs and summarizes the experiments with Simplified Fuzzy ARTMAP network applied to renal failure data.

SFAM is a fast, online/interactive, incremental, supervised learning system for analog signals. It is essentially a two-layer network that is specialized for pattern recognition, capable of learning every training pattern with very little iteration. This translates the processing time measured in seconds rather than days or weeks to run 1000s of epochs in conventional back-propagation neural networks. The network starts with no connection weights, grows in size to suit the problem, uses simple learning equations, and has only user-selectable parameter. SFAM surpasses the performance of many other machine learning techniques in many

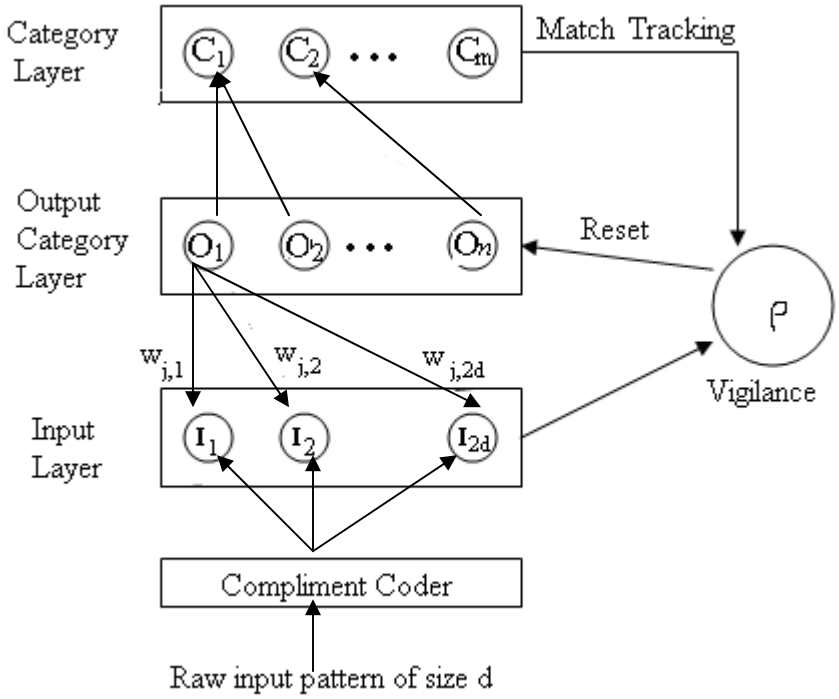
benchmark problems. This fast learning capability is made possible by a series of processing stages: input creation, input formatting output node activation, pattern matching, and categorical mapping.

SFAM contains two layers: an input and an output layer. A block diagram of the SFAM network showing the main architecture is given in Fig.7.1. Training begins with just one hidden node whose weights are set equal to the first record and prediction is set equal to the class of the first record. Similarly, whenever a new class is encountered a new node is created. The node whose weights best match the current input supplies the prediction, provided the degree of the match exceeds the vigilance threshold value. If this prediction is correct, the weights of this winning node are adjusted toward this input. If the prediction is wrong or vigilance threshold is not achieved, a new node is created with weights and prediction equal to this record.

Network is said to be in state of resonance if the network function value exceeds vigilance parameter. Network is said to be in state of mismatch reset if vigilance parameter exceeds match function value. Once the network has been trained by passing input pattern into complement coder and then input layer, all the output nodes compute activation function with respect to input. The winner, which is the node with the highest activation function, is chosen.

Input into the network must be normalized to a value from 0 to1, hence a suitable normalization value must be chosen so that no input will fall outside of the valid range. A compliment coder normalizes the input and also provides the fuzzy compliment for each value. This expanded input (I) is then passed to the input layer. Weights (w) from each output node sample the input layer, making the weighting top-down. The category layer merely holds the names of the (m) categories that the network is expected to classify.

Fig. 7.1: Block diagram of SFAM network



The training algorithm is now described for completeness. For a given input vector a of d features, the compliment vector $\bar{a}$ represents the absence of each feature

$$\bar{a} = 1 - a \tag{1}$$

The internal compliment coded input vector I is then of dimension 2d.

$$I = (a, \bar{a}) = (a_1, a_2, ...a_d, \bar{a}_1, \bar{a}_2, ...\bar{a}_d) \tag{2}$$

The activation and matching functions were defined as

$$T_j = \frac{|I \wedge W_j|}{\alpha + |W_j|} \tag{3}$$

$$M = \frac{|I \wedge W_j|}{|I|} \tag{4}$$

Where Wj are current values of templates a (weight vector) associated with output nodes j and α is a small value close to zero. The updates of templates that belong to resonant domain are represented as an assignment statement.

$$W_j = (1 - \beta)W_j + \beta|I \wedge W_j| \tag{5}$$

Where β is the learning rate, $0 \leq \beta \leq 1$.

The operator $\left|I \wedge W_j\right|$ =∑ min (I, W_j) used in (4) and (5) defines “fuzzy ANN“ which assumes positive, normalized values of the inputs.

Once SFAM has been trained, a ‘feed-forward’ pass through the compliment-coder and into the input layer classifies an unknown pattern. The output node activation is evaluated for each output node in the network. The category of the input is found by assigning it the category of the most highly activated node max (T_j).

Application to renal failure classification: The kidneys are the body’s filtering system. There are many risk factors for renal failure. Loss of renal function may also develop gradually over time, with few symptoms in early stages, referred as chronic renal failure. High blood pressure and diabetes are the most common causes of chronic renal failure. The renal failure data were collected from Government General Hospital, Chennai which comprises of 1200 cases over a period of six years. There were 126 acute, 778 chronic and 296 diabetic renal failure cases respectively. The network model consists of 17 input nodes. The commonly used 2/3 and 1/3 partitions were adopted for the training and testing. The training data was used to train the model, test data was used to measure the performance of the trained network. Out of the 1200 renal failure patients 847 (70.6%) were men and mean age was 48 years. Using the 17 covariates, the aim is to classify the cases into pair wise categories.

Results:

The overall classification of chronic and diabetic categories is given in table 7.1.

Table 7.1: Confusion Matrix (Chronic vs Diabetic)

↓ **Actual/Predicted** →	Chronic	Diabetic	Total	Prediction Error %
Chronic	246	06	252	2.4%
Diabetic	18	88	106	17.0%
Total	264	94	358	6.7%
Actual Error%	6.8%	6.4%	6.7%	

The neural networks will prove useful in renal failure prediction with selected important covariates as inputs. Moreover, SFAM is sensitive to the order of the inputs. To overcome this problem and to increase the classification percentage, we can utilize multiple SFAM networks with a voting scheme. Another limitation of SFAM for medical decision-support applications is it has no provision for missing data items when generating predictions. A variant of fuzzy ARTMAP, known as fusion ARTMAP, has a solution for this problem. The results show that SFAM has superior performance compared to Multilayer perception. A comparative study of SFAM with other techniques like Radial Basis Function, Recurrent networks are in progress.

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8. BIOMEDICAL INFORMATICS CENTRE

Biomedical informatics centre (BIC) has been setup by the Indian Council of Medical Research at TRC during this year. The BIC at TRC has been started

- for further understanding of TB and HIV/AIDS by computational approach
- to provide bioinformatics facilities for biomedical research
- for imparting skills in bioinformatics by organizing training programmes/ workshops and
- to extend the research facilities in bioinformatics for other academic institutions.

Director has appointed a Senior Research Officer, a Research Officer and a Junior Research Fellow. The centre has a server, four clients, a printer, a scanner with an UPS. Two software packages viz., Accelrys Discovery Studio and Accelrys GCG are also available at BIC. Discovery Studio is used for Molecular modeling, Docking and Simulation studies, and GCG for sequence analysis. These bioinformatics software packages would be useful for Scientist and Research scholars of TRC as well as other educational and research institutes. Further, the BIC would also initiate new research projects and collaborate with other research units in the field of TB, HIV/AIDS and other infectious diseases.

Staff working in the biomedical informatics centre

(Contact Person: Mr. S. Jagadish Chandra Bose; sjcbose@trcchennai.in)

9. LIBRARY AND INFORMATION SERVICES

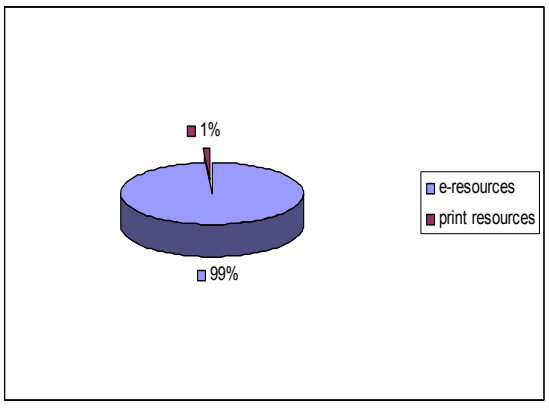
The library and information services of TRC are laid down to gather health information and make it accessible to the scientific community. The library has campus wide electronic access facility for its electronic resource services. It caters to the felt needs of scientific community of ICMR institutes in India.

Digital Library

The digital library is serving as a web-based interface for the in-house resources as well as hyperlink to all the subscribed e-resources and JCCC@ICMR. Pointers have also been given to outside free online resources, viz., medIND; J-Gate; HighWire Press; FreeFullText.com; DOAJ; etc in this platform. The digital library tries to accomplish, make it easier to access the available online international resources.

e-Resources building

The electronic resources have been strengthened with e-bundle; subject collection; cumulative collection; databases and archives.



e-resources Interface

Automation:

Circulation process is being functioned through the software GLAS (Graphical Library Automation System, USA). The Online Public Access Catalog is being made available through local area network (LAN).

Future Plan:

It is planned to introduce RF ID technology for library in-house operations

Electronic wealth:

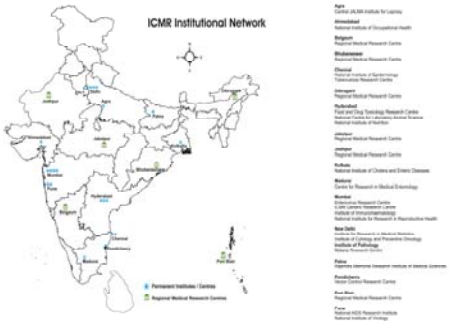
BarCode Printer	1 no.	BarCode Scanner	2 nos.
Laser Printer	1 no.	iMac	4 nos.
Personal Computer	5 nos.	Power Mac G5	1 no.
High-end Scanner	1 no.		
[slide and negative scanning facility]			

Multimedia Unit

Multimedia Unit services include Annual Report co-ordination for designing and compilation, co-ordination for scientific publications and thesis work relating to scanning and editing, Portable Document Format (pdf) conversion for print article, Geographical Information System (GIS) analysis, co-ordination for designing and data management for TRC Web site and poster designing for scientists.

ICMR Institutional Network

ICMR Institutional Network board has been designed and displayed at the front desk of the library to provide information about ICMR network in India to the common public as well as scientists coming from outside.



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10. ELECTRONIC DATA PROCESSING

The Electronic data processing (EDP) division provides computerized services for all departments in the TRC. The different departments have direct access to the data with their personal computers (PC). The EDP division is continuing to give data management support including data entry/verification to various studies undertaken in the Centre. Also, this division generates reports and prepares pre-printed forms for field activity and supply data tabulations for monitoring the studies and publication of research work. The EDP division completed the process of data entry and analyzed the data for the mortality survey conducted in Orissa and Andhra Pradesh states during the year.

Data entry and data management training were provided to the staff of RMRC, Jabalbur and MGIMS, Wardha for the “Prevalence of TB survey” during the year.

The key requirements viz., data entry, information process and e-mailing has been maintained well. This division helps in providing audio-visual system for the presentation of research materials whenever conferences, meetings and trainings are being held.

The existing LAN facility has been well utilized by the researchers, students and trainees. The wireless internet facility was provided in the main building of TRC and at Madurai unit. Much care has been taken to prevent the attack of viruses by updating the anti-virus software in the network server at all times and checking all incoming e-mail for viruses.

This division helped in setting up the newly formed ICMR - Biomedical informatics centre (BMIC) under the guidance of the Director, TRC. For this purpose, ICMR has provided the equipments such as a network server, a laser printer, a scanner, four numbers of PIV desktop computers and a 5 kva UPS system.

Almost all of the break-down calls of computers and its peripherals were dealt under comprehensive annual maintenance contract. This includes managing the installation of the facilities and ensuring that the computers are maintained and kept up to-date. In this division, at present, five Data entry/verification operators, five Data processing assistants, one Network coordinator and one EDP-In charge are working.

The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April 2006 to March 2007 is shown below.

No. of documents entered: 2,99,074

No. of documents verified: 2,55,644

A total of 1, 61,284 records were processed for the on-going second and third resurveys of Disease survey conducted at Tiruvallur district. Twenty two panchayats' pre-printed cards and 24 panchayats' person's alphabetical name-wise and household-wise lists were supplied for the third resurvey. The second resurvey data clean work has been taken for data analyses.

Data processing staff working in the EDP division

Data entry staff working in the EDP division

(Contact Person: Mr.R.Subramani; subramanir@trcchennai.in)

11. ICER Project

Establishment of an International Centre of Excellence in Research at the Tuberculosis Research Centre

The Tuberculosis Research Centre and National Institutes of Health (NIH), USA, have been collaborating on several aspects of infectious diseases especially in the field of filariasis for several years. This long standing collaboration has now allowed the setting up of an International Centre of Excellence in Research (ICER) at TRC (only one of 3 such centres all over the world). The proposal for the establishment of this centre was jointly submitted by Laboratory of Parasitic Diseases of NIAID/NIH, USA and ICMR. The proposal was then chosen for funding from among several proposals submitted by many centres.

The following are the objectives of this project:

1. Establishment of an International Centre of Excellence in Research on infectious and allergic diseases jointly by the Indian Council of Medical Research and the National Institute of Health at the Tuberculosis Research Centre, Chennai.

2. Strengthening of the infrastructure for basic science and product development oriented research in infectious and allergic diseases at the Tuberculosis Research Centre. This infrastructure development will be aimed at establishing research facilities that would provide the Centre the tools of modern biology particularly in the areas of genomics, proteomics and bioinformatics to study these diseases.

3. Promoting collaborative research in infectious and allergic diseases between the Indian Council of Medical Research and the NIH in particular and other Indian and international scientists in general.

4. Encourage capacity building by providing training in clinical and laboratory skills to both Indian and foreign scientists in infectious and allergic diseases.

This unique project is funded totally out of NIH intramural funds is designed to develop infrastructure and build the capacity for international collaborative research. Under the aegis of the collaboration a state of the art fully equipped modern biology lab has been established at TRC.

12. MISCELLANEOUS

RNTCP Training:

Good quality is essential for the successful implementation of any programme. Therefore, TRC has been identified as nodal Centre for training in RNTCP. During the training period 2006-2007, 57 Medical Officers (Tr), 20 Medical Officers, 58 Senior Treatment Supervisors, 14 Senior TB Laboratory Supervisors and 9 Lab Technicians have been trained. State TB Officer and 27 District TB Officers have also been trained on Pediatric-wise Drug Box for one day.

Training in TB prevalence/ ARTI survey:

Our centre organized two training programmes; one to the project staff of RMRC, Jabalpur in conducting a TB prevalence /ARTI survey among Tribals in Madhya Pradesh and another to the project staff from MGIMS, Wardha in conducting a prevalence survey based on symptom and X-ray in Wardha district. First, a team consisting of census takers, sputum collector, tuberculin testers and readers from RMRC was given training in the field unit, Tiruvallur on survey methodology like door-to-door census, registration, and symptom elicitation, sputum collection tuberculin testing and reading for a period of about three weeks. The Laboratory technician was trained in sputum microscopy, culture of mycobacteria, and identification and drug susceptibility tests in the laboratory of the Bacteriology department of the centre. The Research Assistant was given training in the Statistics Division in scrutiny of data, arranging the documents for data entry, preparing the format for data entry, data management and analysis. The Data entry operators were trained in computerization of data, verification and maintenance of the computerized data by the EDP Division. Apart from giving training at TRC, a team of field staff with a higher official visited the study area in MP and conducted a reorientation training programme for a week.

Similar training was given to another team of project staff from Wardha in survey methodology. Since, X-ray chest radiograph is also a screening tool for this survey, three X-ray readers were given training in reading the MMR films at TRC on two occasions.

TRC Annual Day Celebrations

The TRC Annual Day was celebrated on 7th December 2006 at Chennai and Thiruvallur and on 13th December at Madurai. A total of 123 staff members of the TRC who had completed 25 years of meritorious service were honoured on this occasion by being presented with a wrist watch. The director, Dr. P R Narayanan presided over functions at all three sites and handed over the memento to the staff members.

3100 – Avant Genetic Analyzer

This is an upgradeable four capillary ABI – Prism Genetic Analyzer which can be used for standard sequencing analysis with a 98.5 base calling accuracy as well as single nucleotide polymorphism identification by 5 color fluorescence up to 100 bases.

APPENDICES

Publications

Publications in	i)	International journals	: 33
	ii)	National journals	: 26
Accepted for publication in	i)	International journals	: 12
	ii)	National journals	: 6
	iii)	Books	: 4

Published

International:

1. Babu S, Blauvelt CP, Kumaraswami V, Nutman TB. Cutting Edge: Diminished T cell TLR expression and function modulates the immune response in human filarial infection. J Immunol. 2006;176(7):3885–3889.
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International:

1. Geetha Ramachandran, Hemanthkumar AK, Rajasekaran S, Padmapriyadarsini C, Narendran G, Anitha S, Kumaraswami V, Soumya Swaminathan. Steady state pharmacokinetics of nevirapine in HIV-1 infected adults in India. J Intl Assoc Phy AIDS Care.
2. Gomathi NS, Sameer H, Vanaja Kumar, Balaji S, Azger Dustackeer VN, Narayanan PR. *In silico* analysis of mycobacteriophage Che12 genome: characterization of genes required to lysogenise *Mycobacterium tuberculosis*. Computational Biology and Chemistry.
3. Lakshmi S, Ramesh K, Valerie Jauvin, Marie Helene Schrive, Ranjani Ramachandran, Narayanan PR, Fleury HJ, Soumya Swaminathan. Characterization of HIV-1 Isolates from Anti-retroviral Naive Children in South India. AIDS Res Hum Retroviruses.
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5. Natarajan PL and Sujatha Narayanan. Role of mitogen activated protein kinase and *N*-acetyl-*L*-cysteine in mediating *Mycobacterium tuberculosis* H37Rv-induced interlekin-6 secretion in THP-1 human monocytes FEMS Immunol Med Microbiol.
6. Ramalingam B, Alain R. Baulard, Camille Locht and Alamelu Raja. Antibody response in pulmonary tuberculosis against recombinant 27kDa (MPT51, Rv3803c) protein of *Mycobacterium tuberculosis.*Scand. J Infect Dis.
7. Soumya Swaminathan, Padmapriyadarsini C, Ranjani Ramachandran, Pradeep A Menon, Nalini S Mohan, Kubendiran G, Paramasivan C.N. Localized Pulmonary Mycobacterium avium Disease in Acquired Immunodeficiency Syndrome. J Int Assoc Phy AIDS Care.
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10. Sujatha Narayanan, Sebastien Gagneux, Lalitha Hari, Anthony G. Tsolaki, Suganthi Rajasekhar, Narayanan PR, Peter M. Small, and KathryDeRiemer, Genomic interrogation of ancestral *Mycobacterium tuberculosis* from South India. Infection, Genetics and Evolution.

11. Sulochana S, Narayanan S, Paramasivan CN, Narayanan PR. Analysis of Fluoroquinolone resistance in clinical isolates of *Mycobacterium tuberculosis* from India. J. Chemother.
12. 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.

National:

1. Geetha Ramachandran, Hemanth Kumar AK, Sarala K, Padmapriyadarsini C, Anitha S, Tharani CB, Kumaraswami V, Soumya Swaminathan. Urinary levels of isoniazid and rifampicin in asymptomatic HIV-positive individuals. Ind J Med Res.
2. Jaggarajamma K, Sudha Ganapathy, Chandrasekaran V, Nirupa Charles, Thomas A, Santha T, Muniyandi M, Narayanan PR. Reasons for non-compliance among TB patients treated under RNTCP in Tiruvallur District, south India. Ind J Tub.
3. Raghu B, Venkatesan P. Effect of n-3 Fatty acid supplement on blood glucose, lipid profile and cytokines in humans. Ind J Clin Biochem.
4. Soumya Swaminathan, Narendran G, Pradeep A. Menon, Padmapriyadarsini C, Arunkumar N, Sudharshanam NM, Ramesh Kumar S, Chandrasekhar S. Impact of HIV infection on radiographic features in patients with pulmonary tuberculosis. Ind J Chest Dis All Sci.
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. Ind J Tub.
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. Ind J Tub.

Books: (in press)

1. Vallinayagam V, Senthamaraikkannan K, Venkatesan, P. A Markov Chain Monte Carlo Algorithm from multiple imputation of missing values. In **Computing and Mathematical Modeling** (Ed. P. Balasubramaniam and R. Uthayakumar) Allied Publishers.
2. Venkatesan P, Porchelvan S. AIDS projection using non-parametric back calculation method. In **Computing and Mathematical Modeling** (Ed. P. Balasubramaniam and R. Uthayakumar) Allied Publishers.
3. Venkatesan P, Suresh M.L. Neural network model for classifying renal failure. In **Computing and Mathematical Modeling** (Ed. P. Balasubramaniam and R. Uthayakumar) Allied Publishers.

4. Rathinasabapati R. Designing digital architecture with intelligent building for information access at the Tuberculosis Research Centre, Chennai *Source*: **International Conference on Semantic Web and Digital Libraries –ICSD 2007**, Edited by A.R.D Prasad & Devika P. Madalli, p.357. (Article available at the DRTC Instgitutional Repository: (http://drtc.isibang.ac.in/DRTC/index.html)

Awards / Honours

- **"RC Garg Memorial Award"** for the best article published in Indian Journal of Tuberculosis for the year 2005: Article title: Is it worth treating Category I failure patients with Category II regimen? – **Dr.T.Santha**
- **"Prof. KC Mohanty Award"** for the best paper presented at the 60th National Conference on Tuberculosis and Chest Diseases held at Lucknow in February 2006. The paper is entitled "A new measurable indicator for Tuberculosis (TB) case detection in Revised National TB control programme" – **Mr. P.G.Gopi**
- Elected as "**Fellow of Indian Society for Medical Statistics-FSMS**" and the Award was given in Nov 2006 - **Dr. P. Venkatesan**
- **"Prof. BG Prasad Award"** for the best paper published on Statistics in the field of Epidemiology in the journal 'Statistics in Medicine' for the year 2005 at the Annual Conference of the Indian Society for Medical Statistics held at Belgaum during Dec. 2006 – **Dr.V.Chandrasekaran**
- **"Lifetime Achievement Award"** for promoting knowledge and research in Applied Microbiology by the Indian Association of Applied Microbiology at Kancheepuram during Jan. 2007 - **Dr.N.Selvakumar**
- **"Prakash Award"** for the best poster presentation at the 4th Convention of Society for Immunology and Immunopathology (SIIP) and National Symposium on immunobiotechnology held at Chennai during Feb. 2007 – **Ms.Shenbagavalli**

PARTICIPATION IN CONFERENCES / SEMINARS / WORKSHOPS / TRAINING PROGRAMMES

1. **Clinical Research and Clinical Trials** held at Mumbai during Apr. 2006 – Aleyamma Thomas, Soumya Swaminathan, Pauline Joseph.

2. **Keystone Symposia** in Keystone, Colorado, USA during Apr. 2006 – Soumya Swaminathan

3. **Sensitisation Workshop on "Pediatric Patient–wise boxes"** held at Bangalore, during Apr. 2006 – Rajeswari Ramachandran

4. **National Conference on Research in HIV and AIDS** held at New Delhi during Apr.2006 – Soumya Swaminathan

5. **Indo-Swedish (Karolinka Institute) symposium** on Genomics and Proteomics of Diabetes held at Chennai during Apr. 2006 – P. Venkatesan.

6. **Workshop on "Analysis in Qualitative Research"** held at Chennai during Apr. 2006 – Mohanarani Suhadev.

7. **Symposium on "Method Development and Beyond"** held at Chennai during 2006 – Gomathi Sekar.

8. **Training on Qualitative Research Methodology** at University of Alabama at Birmingham, (UAB) USA during Jan - May 2006 under the TRC/ICER programme. - Meenalochani Dilip

9. **Clinical Development Workshop** held at Singapore during May 2006 – M.S. Jawahar.

10. **Product Development Meeting** held at Bangalore during May 2006 – M.S. Jawahar

11. **Sensitisation meeting for Medical Officers of NGOs** held at Chennai during May 2006 – M.S. Jawahar.

12. **Training program in "Design and Conduct of Clinical Trials"** held at University of Alabama, Birmingham, California and in Bethesda during May-Aug. 2006. – G. Narendran.

13. **"Workshop on Statistical Techniques for Micro array Data Analysis"** held at Chennai during Jun. 2006 - P. Venkatesan

14. **Training on application of EPICS XL / MCL Flow Cytometer** held at Thiruvanthapuram during Jun. 2006 – S. Murugesan.

15. **Indo-US Workshop on Bioethics in Clinical Research** held at New Delhi during Jun 2006 – Soumya Swaminathan.

16. **1st SAARC Seminar on Pediatric HIV** held at Mumbai during Jun 2006 – Soumya Swaminathan.

17. **International Conference on "Measuring progress towards MDGs for TB control"** held at Geneva during Jun 2006 – P.G.Gopi.

18. **Workshop on "Communication Skills"** held at Chennai during June 2006 – M. Senthilkumar

19. **Workshop on "Future ARTI Surveys in India"** held at Bangalore during July 2006 – P.G.Gopi.

20. **Scientific writing workshop** held at Chennai during July 2006 – Ranjani Ramachandran, C. Padmapriyadarsini, Sulochana Das, V.V.Banurekha, Sheik Ilias, Geetha Ramachandran, Jaggarajamma, Thiruvalluvan, Kaustav Nayak.

21. **Workshop on "Good Clinical Practice for HIV Vaccine Trial"** held at Chennai during July 2006 - Sudha Ganapathy.

22. **Zonal Task Force meeting of Medical Colleges** held at Hyderabad during Aug. 2006 – Aleyamma Thomas.

23. **Workshop on "Tuberculosis Surveillance and Monitoring"** held at Bangalore during Aug. 2006 – P.G. Gopi.

24. **National IMA Workshop on "RNTCP Public Private Mix–DOTS (PPM-DOTS)"** held at New Delhi during Aug. 2006 – Aleyamma Thomas.

25. **WHO Workshop on "Surveillance of Drug Resistance"** held at Pune during Aug. 2006 – K. Ramesh.

26. **Continuing Medical Education 2006** held at Kurnool during Aug.2006 – M.S. Jawahar

27. **16th International AIDS Conference** held at Toronto, Canada during Aug. 2006 – Soumya Swaminathan.

28. **AIDS Vaccine 2006 conference** held at Amsterdam during Aug-Sept. 2006 – Mohanarani Suhadev.

29. **2nd Medical Development Congress** held at New Delhi during Sept. 2006 – Sujatha Narayanan.

30. **Emerging and Re-emerging Infectious Diseases and Disease Surveillance** held at New Delhi during Sept. 2006 – N. Selvakumar

31. **Maternal and Early childhood infections: Interplay, prevention and management** held at New Delhi during Sept. 2006 – Soumya Swaminathan

32. **RNTCP Zonal Task Force Meeting** held at Ahmedabad during Sep. 2006 – Rajeswari Ramachandran.

33. **Workshop on "Operational Research Methodology"** held at Chennai during Sept. 2006 – Aleyamma Thomas, Rajeswari Ramachandran, Rani Balasubramanian, Pauline Joseph, P.G.Gopi, Sudha Ganapathy, Nirupa Rani Charles, Jagga Rajamma, M. Muniyandi, V. Chandrasekaran.

34. **17th CME of Dr. Athavale Research Foundation** held at Goa during Sept. 2006 – Soumya Swaminathan.

35. **Training on "Analysis of HIV-1 resistance to antiretroviral drugs by sequencing"** held at France during Sept. 2006 – S. Lakshmi.

36. **Workshop on "Nursing Administration and Supervision for effective patient care"** held at New Delhi during Sept. 2006 B.V.S.C.Rao, G.Mangalambal.

37. **International Joint Monitoring mission for evaluation of RNTCP** during Oct. 2006 – Aleyamma Thomas.

38. **National Symposium on Tribal Health** held at Jabalpur during Oct. 2006 – P.G. Gopi, R. Subramani, G. Narendran, Kaustav Nayak.

39. **South Zone Conference of Indian Psychiatric Society** held at Mangalore during Oct. 2006 – Pradeep Menon

40. **9th National Conference of Pediatric Infectious Diseases** held at Chennai during Oct. 2006 – Soumya Swaminathan.

41. **Workshop on "Systematic Review"** held at New Delhi during Oct. 2006 – M.S. Jawahar

42. **Workshop on "Statistics in Clinical Research"** held at Mohali during Oct. 2006 **–** A.K. Hemanthkumar, L. Sekar, B. Sugumar

43. **1st Expert Group Meeting for setting up of National Clinical Trials Registry** held at New Delhi during Oct. 2006 – M.S. Jawahar.

44. **8th International Conference on Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases (MEEGID – VIII)** held at Bangkok, during Nov. 2006 – Sujatha Narayanan.

45. **37th Union World Conference on Lung Health and Supra-National Laboratory Meeting** held at Paris during Nov. 2006 – N. Selvakumar

46. **CME in Biostatistical Techniques in clinical trials in the XXIV ISMS conference** during November, 2006 at Coimbatore – P.Venkatesan

47. **27th Annual Conference of Indian Association of Biomedical Scientists (IABMS)** during Nov. 2006 – K.V.Kuppurao.

48. **National Task Force for Medical College - Involvement in RNTCP** – held at New Delhi during Nov. 2006 - Pauline Joseph

49. **Workshop on "Total Quality Management"** held at Kalpakkam during Nov. 2006 – M. Senthilkumar

50. **Continuing Medical Education for Medical Colleges on RNTCP** held at Thiruvanthapuram during Dec.2006 – Rajeswari Ramachandran.

51. **24th Annual National Conference of ISMS held at Coimbatore** during Dec. 2006 – M. Vasantha

52. **Workshop on "Clinical Biostatistics and Research Methodology"** during Dec. 2006 at the SRM University, Chennai – P.Venkatesan.

53. **International Conference on Reliability and Safety Engineering (INCRESE 2006)** held at Chennai during Dec. 2006 - P. Venkatesan.

54. **33rd Indian Immunology Society Conference** held at New Delhi during Jan. 2007 – Sujatha Narayanan, P. Supriya, P. Rajashree, Kaustav Nayak.

55. **5th Annual Conference of Indian Association of Applied Microbiologists** held at Kancheepuram during Jan. 2007 – N. Selvakumar, Vanaja Kumar, Gomathi Sekar, N.S. Gomathi, Azger Dusthakeer, S. Balaji

56. **Continuing Medical Education for Medical Colleges on RNTCP** held at Coimbatore during Jan.2007 – Rajeswari Ramachandran.

57. **Training Programme on DOTS Plus** held at New Delhi during Jan. 2007 – Rajeswari Ramachandran.

58. **National Lutheran Health & Medical Board Meeting for Pastor's training on "Human Sexuality and Church Response"** held at Chennai during Jan. 2007.

59. **National Conference of Indian Psychiatric Society** held at Chennai during Jan. 2007 – Pradeep Menon

60. **HIV/AIDS awareness programme** held at Chennai during Jan. 2007 – Beena E. Thomas.

61. **International Conference on "The Role of Not for Profit Organizations in the Development of Health Care"** held at Chennai during Jan. 2007 – R. Balambal.

62. **National Seminar on "Information literacy and Higher Education"** held at Chennai during Jan. 2007 – R. Rathinasabapathi.

63. **National Workshop on Microarray Data Analysis** held at Chennai during Feb. 2007 – P. Venkatesan

64. **Regional Workshop on "Greenstone Digital Library Software"** held at Vellore during Feb. 2007 – R. Rathinasabapathi

65. **Short course Training on Statistical Analysis System (SAS)** held at Christian Medical College, Vellore during Feb. 2007 -V. Chandrasekaran

66. **International Conference on Semantic Web and Digital Libraries** held at Bangalore during Feb. 2007 – R. Rathinasabapathi

67. **14th Conference on "Retroviruses and Opportunistic Infections"** held at Los Angeles, USA during Feb. 2007 – C. Padmapriyadarsini, Geetha Ramachandran

68. **61st National Conference on "Tuberculosis and Chest Diseases"** held at Udaipur during Feb. 2007 - N. Selvakumar (Chairperson), Beena E. Thomas, Jaggarajamma, A.K.Hemanthkumar, N.S.Gomathi, Gomathi Sekar, Azger Dusthakeer.

69. **4th Convention of the Society for Immunology and Immunopathology in Chennai** held at Chennai during Feb. 2007 – V.D. Ramanathan, P. Selvaraj, P. Supriya, P. Rajashree, Nisha Rajeswari, M. Vidya Rani, K. Alagarasu.

70. **CME program of Indian Association of Medical Microbiologist** held at Chennai during Feb. 2007 – Vanaja Kumar, Nalini Sundar Mohan, S. Balaji

71. **GFATM meeting** held at New Delhi during Mar. 2007 – Rajeswari Ramachandran.

72. **Review Meeting on "Mw Vaccine Study"** held at New Delhi during Feb. 2007 – Rajeswari Ramachandran.

73. **Continuing Medical Education Programme on RNTCP** held at Ludhiana and Amritsar during Feb. 2007 – Rajeswari Ramachandran, R. Balambal.

74. **State Level Seminar on "Social Work Research in the Indian Context"** held at Chennai during Feb. 2007 – Beena E. Thomas.

75. **Workshop on "Qualitative Research Methodology"** held at Chennai during Feb. 2006 – Beena E. Thomas, Nirupa Rani Charles, Meenalochini Dilip, Sarala, Gunasundari, Shyamala.

76. **Workshop on "Research Dissemination"** held at Madurai during Feb.2007 – M.S.Jawahar, Soumya Swaminathan, Geetha Ramachandran, G.Narendran, Gunasundari, C.Ponnuraja, Mohanarani Suhadev.

77. **UGC-SAP National Conference on Mathematics, Computing and Modeling** held at Gandhigram during Mar. 2007 - P. Venkatesan.

78. **Workshop on Model-based HIV estimation for Southern Region** held at Chennai during Mar. 2007 - P. Venkatesan.

79. **National Seminar on Recent Advances in Biostatistical Methods** held at Chennai during Mar. 2007 – P. Venkatesan.

80. **Continuing Medical Education 2007** held at Chennai during Mar 2007 – M.S. Jawahar.

81. **Indo-Australian conference on "Human variations and Pharmacogenomics** held at Manipal during Mar. 2007 – Geetha Ramachandran, A.K. Hemanthkumar.

82. **Seminar on 'HPLC – Trouble shooting tips"** held at Chennai during Mar. 2007 – Geetha Ramachandran, A.K. Hemanthkumar.

83. **National Level Technical Symposium** held at Chennai during Mar.2007 – Sujatha Narayanan.

84. **National Symposium on "Recent immunological advancement in HIV and Opportunistic infections"** held at Chidambaram during Mar. 2007 – Sujatha Narayanan.

85. **National seminar on "Recent Trends In Laboratory Diagnosis of Bacterial Infections"** held at Chennai during Mar. 2007 – Sujatha Narayanan, G. Kubendiran.

86. **DBT HIV Meeting - "HIV/AIDS: Biology, Immunology and Vaccinology"** held at New Delhi during Mar.2007 – Soumya Swaminathan.

87. **Workshop on "Fundamentals of Biostatistics in Clinical Research"** held at New Delhi during Mar. 2007 – Soumya Swaminathan.

88. **Symposium on "Challenges and Research Opportunities"** held at Los Angeles during Mar 2007 – C. Padmapriyadarsini.

89. **Workshop on "Teaching Listening Skills"** held at Chennai during Mar. 2007 – M.Senthilkumar.

WORKSHOPS / CONFERENCES / SEMINARS / TRAINING PROGRAMME CONDUCTED AND ORGANIZED BY THE INSTITUTE

Workshop :

1. Research Dissemination Workshop: **Impact of Nutritional Intervention in HIV Positive Persons** on 2nd March 2006, Chennai (HIV Dept.) The importance of nutrition and nutritional counselling in improving the clinical condition, laboratory parameters as well as the quality of life in PLWHA were depicted in various presentations done by TRC staff.
2. **Scientific writing** workshop organized jointly by TRC/UAB held at Chennai during July 2006
3. Tuberculosis Research Centre (ICMR) and World Health Organization Jointly Organized an **Operational Research Methodology** Workshop at Chennai between 18 - 20 September, 2006.
4. **Research Dissemination** Workshop at TRC Madurai on 2nd February 2007 (HIV Dept.) An overview of each study and its interim findings were presented by the TRC clinical staff. A brief note of the clinical trials and procedures in research as well as the role of each of the participants in improving recruitment and increasing interaction were discussed.
5. A TRC/UAB/ICER sponsored Workshop on "**Qualitative Data Analysis and Interpretation** ." was conducted at TRC from Feb12th -16th 2007 by Dr.Joseph Telfair Dr. PH, MSW, MPH Professor, Public Health Research and Practice,Department of Public Health Education , University of North Carolina at Greensboro.USA. (Clinic Dept.) Qualitative Research Methods, Basics of Data Analysis, Interpretation of Focus Group Data and practical work on steps in Focus Group Data Analysis and Interpretation were covered during the workshop.
6. State Level Workshop on pediatric HIV/AIDS for Tamilnadu 3rd September 2006.
7. Collaborators Meeting on 27th July 2006.

SPECIAL ASSIGNMENTS

Dr. N.Selvakumar

- Training of trainers of SAARC countries at Paro, Bhutan. SAARC TB and HIV/AIDS centre, Nepal and Kingdom of Bhutan. 17-24 June 2006. (IUATLD- Facilitator).
- WHO-TRC Regional Consultation on laboratory expansion in control of tuberculosis, Chennai. 11-13 July 2006. (WHO Temporary Advisor).
- Review of Mycobacteriology laboratory in RMRC-T (ICMR) Jabalpur. 18-20 July 2006. (National Expert).
- WHO-CTD Review meeting of RNTCP consultants and STOs. Chennai. 23 July 2006. (National Expert).
- Strengthening of EQA of sputum AFB microscopy. Visit to Uttar Pradesh. Aug 27 2006. (IUATLD regional advisor).
- Regional training course on Quality Assurance for sputum Microscopy for TB Control. New Delhi TB Centre. LRS Institute, New Delhi. 1-7 Sep 2006. (IUATLD - Guest - Facilitator).
- Review of Mycobacteriology laboratory in RMRC (ICMR) Dibrugarh. 9-11 Jan 2007. (National Expert).
- Laboratory Committee member, RNTCP, CTD, New Delhi. (2002-till date).

Dr.V.D.Ramanathan

- Histopathology Consultant for Chingleput Leprosy Teaching and Research Institute

Dr. Alamelu Raja

- Expert Member of the Institutional Review Board of Sri Kanchi Kamakoti HILDS Trust Hospital
- Member, Editorial Board, Indian Journal of Medical Research.

Dr. Sujatha Narayanan

- Doctoral committee member for two Ph D students from the Department of Biotechnology , Anna University, Chennai
- Thesis and Viva Voce examiner for a Ph D student in the Indian Institute of Science, Bangalore

Dr. P.Selvaraj

- Executive council member, Indian society for histocompatibility and immunogenetics, New Delhi
- Reviewer for Journals:
  - **International**

    i) Am J Resp Crit Care Med
    ii) Tuberculosis
    iii) J Clin Immunol
    iv) Int J Tuberc Lung Dis
    v) Nanotechnology
    vi) Nephron

  - **National**

    i) Ind J Exp Biol

- Extgernal Examiner for M.Sc., Theory and Practical exams for M.Sc., (Human Genetics) and M.Sc., (Biotechnology) at Sri Ramachandra University, Chennai

Dr. P. Venkatesan

- Elected as “Fellow of Indian Society for Medical Statistics-FSMS” and the Award was given in Nov 2006.
- Served as Expert Member Inspection Committee by the Tamilnadu Dr MGR University, Chennai for granting affiliation to M.Sc. (Biostatistics) Course at Christian Medical College, Vellore.
- Expert Member- Scientific Advisory Board, Sai’s Biosciences Research Institute, Chennai
- Chairman- Board of Studies-MSc (Bioinformatics)-Sri Ramachandra Medical University, Chennai.
- Member-Board of Studies- MSc (Statistics)- University of Madras
- Member- Board of Studies-MSc (Human Genetics) & BSc (Biomedical Sciences). Sri Ramachandra Medical University, Chennai.
- Member- Board of Studies-MTech (Bioinformatics) and BTech (Bioinformatics), Sathyabama University, Chennai
- .External Examiner-University of Madras, Tamilnadu Dr.MGR Medical University and Sri Ramachandra University, Chennai.
- As External examiner evaluated two PhD theses for the award of PhD Degree for Karnataka University and National Institute of Mental Health and Neurosciences,

- Member- Editorial Board: Journal of Pure and Applied Spectrophysics.
- General Secretary- Indian Society for Medical Statistics (ISMS).
- Member-Executive Committee: International Biometric Society (IR).

Dr. Ranjani Ramachandran

- Member National Laboratory Committee for Mycobacteriology Labs Central TB Division
- Member National DOTS Plus Committee
- Member State Crisis Group for the Govt of Tamilnadu

Dr. Geetha Ramachandran

- Resource person for the HIV fellowship program held at YRG Care, Chennai

Dr. A.K. Hemanth Kumar

- Member of the purchase committee of The Tamil Nadu Dr.MGR Medical University, Chennai for purchase of HPLC system

Ph.D. Scholars

List of staff / students who have got 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. L. Prabhakaran	Isolation, characterization and construction of luciferase reporter phage for diagnosis of *M.tuberculosis*	Dr. P.R. Narayanan
2.	Mr. Deepak Jayakumar	Cloexp Cloning, expression and characterization of a serine threonine protein kinase, *PknE* of *M.tuberculosis* $H_{37}Rv$	Dr. Sujatha Narayanan
3.	Ms. G. Radha	Cloning, expression and characterization of a serine threonine protein kinase, *PknI* of *M.tuberculosis* $H_{37}Rv$	Dr. Sujatha Narayanan

List of 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	Submitted during
1.	Ms. R. Priya	Apoptosis of human monocytes and macrophages by *M.tuberculosis* and its implications on cell mediated immune response.	Dr. Sulochana Das	October 2006
2.	Mr. P.G. Gopi	Impact of DOTS on some of the epidemiological parameters of tuberculosis over a period in a selected district of Tamil Nadu	Dr. B.N. Murthy	January 2007

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

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. degree	Supervisor/Guide
4.	Mr. S. Basirudeen	ICMR	Interferon gamma assay for latent TB infection in HIV patients	Dr. Alamelu Raja
5.	Ms. S. Priya	ICMR	A study of natural killer cells in HIV-TB co-infected patients during and after treatment	Dr. Alamelu Raja
6.	Ms.S. Lakshmi	ICMR	HIV drug resistance	Dr.P.R.Narayanan
7.	Mr. Kaustuv Nayak	ICMR	Evaluation of cellular immune response to infection with HIV-I C subtype in South India	Dr. P.R.Narayanan
8.	Ms. G. Shenbagavalli	ICMR	Serum and tissue complement profile in tuberculosis	Dr. V.D.Ramanathan
9.	Mr. S. Manivannan	CSIR	The role of complement activation and antibody in the early interaction of *M.tuberculosis* and macrophages	Dr. V.D.Ramanathan
10.	Mr.V.Narayana Rao	CSIR	Complement activation by strains of *mycobacteria* wild type and gene disrupted *M.tuberculosis* and recombinant BCG	Dr.V.D.Ramanathan
11.	Ms.A. Nusrath Unissa	ICMR	Molecular studies on Isoniazid resistance in *Mycobacterium tuberculosis*	Dr. N. Selvakumar
12.	Ms. Nisha Rajeswari	ICMR	Influence of HLA-DR antigens on immune functions in pulmonary tuberculosis	Dr. P. Selvaraj
13.	Ms. M. Vidya Rani	ICMR	Regulatory role of vitamin D receptor gene polymorphism on cytokine response in pulmonary tuberculosis	Dr. P.Selvaraj
14	Mr. K. Alagarasu	ICMR	Gene polymorphism studies on Chemokine, Chemokine receptors, Vitamin D Receptor (VDR) and Mannose binding lectin (MBL) gene in HIV and HIV-TB patients	Dr. P. Selvaraj

Sl.No.	Name of the candidate	Source of Funding	Title of the Ph.D. degree	Supervisor/Guide
15.	Mr. S.Raghavan	ICMR	Human Leucocycte Antigen (HLA) polymorphism studies in HIV and HIV-TB patients	Dr. P. Selvaraj
16.	Mr. S. Prabhu Anand	CSIR	Regulatory effects of vitamin D_3 and vitamin D Receptor (VDR) genotypes on VDR expression and cytokine production in pulmonary tuberculosis	Dr. P. Selvaraj
17.	Dr. P.L. Natarajan	CSIR	Cellular immunology of TB and HIV/TB	Dr. Sujatha Narayanan
18.	Ms. Harini Laxminarayan	UGC	Study on Molecular Biology of *Mycobacterium tuberculosis*	Dr. Sujatha Narayanan
19.	Ms. Aparna J Christy	ICMR	Development of epitope delivery system for construction of recombinant BCG vaccine for tuberculosis	Dr. Sujatha Narayanan
20.	Ms. V. Malini	ICMR	Functional characterization of FtsY, a signal recognition particle receptor from *M.tuberculosis*	Dr. Sujatha Narayanan
21.	Ms. S. Suba	Corporate Funding	Characterization of the lipoproteins of *M.tuberculosis*	Dr.Sujatha Narayanan
22.	Ms. Neema Bourai	CSIR	Functional characterization of serine/threonine protein kinase 1 of *M.tuberculosis*	Dr. Sujatha Narayanan
23.	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
24.	Ms. C. Prabha	ICMR	Immune response in tuberculosis: TH1/TH2 paradigm	Dr. Sulochana Das
25.	Ms. P. Supriya	CMR	Role of chemokines in tuberculous immunity	Dr. Sulochana Das
26.	Ms. P. Rajashree	ICMR	Role of Dendritic cells in tuberculous immunity	Dr. Sulochana Das
27.	Ms. N.. Yamuna	UGC	Classification and regression trees	Dr. P. Venkatesan

Staff registered (part-time) for their Ph.D. programme at 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. Gomathy Sekar	Optimizing sputum microscopy to detect AFB	Dr. N. Selvakumar
3.	Ms. Mohanarani Suhadev	Sociological aspects of HIV/AIDS	Dr. Udaya Mahadevan
4.	Ms. N.S. Gomathi	Rapid diagnosis and drug susceptibility testing of *M.tuberculosis*	Dr. Vanaja Kumar
5.	Mr. V.N. Azgar Dusthackeer	Mycobacterial latency and tuberculosis diagnosis	Dr. Vanaja Kumar
6.	Mr. Sameer Hassan	Genome analysis of phages and viruses	Dr. Vanaja Kumar
7.	Mr. C. Ponnuraja	Frailty models	Dr.P.Venkatesan
8.	Mr. N. Arunkumar	Causal Analysis	Dr. P. Venkatesan
9.	Mr. B. Sukumar	Statistical Methods for Microarray Data Analysis	Dr. P. Venkatesan

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