Tuberculosis Research Centre

Chennai – 600 031

Research Activities 2005-2006

WHO Collaboration Centre for Tuberculosis Research & Training

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PREFACE

Fifty years have passed since Tuberculosis Research Centre (TRC) was established. During the first two decades, the centre focused all its attention on chemotherapy of tuberculosis and mycobacteriology that resulted in extraordinary results that shaped the management of tuberculosis in the world. In the third decade TRC's participation in Indian Council of Medical Research (ICMR's) Bacillus Calmette Guerin (BCG) trial helped the Govt. of India shaping its policy on BCG vaccination in the country. The attention of TRC in the fourth decade was on establishing the ground rules for management of extra-pulmonary tuberculosis. In the fifth decade TRC joined hands with central TB division of Ministry of Health, Govt. of India, in strengthening Revised National Tuberculosis Control Programme (RNTCP) in the country. In the last few years TRC strengthened the infrastructure for basic research, initiated HIV-TB related studies, established HIV vaccine trial center and NIH center for excellence in research.

Placed against the backdrop of the national and global TB control program, it is heartening to look back and feel proud that TRC has always addressed issues in research that has direct implication in benefiting the TB community. We have made the best use of existing tools for the control of Tuberculosis (TB). The progress of the basic research towards developing newer drugs, diagnostics and vaccines has been less dramatic and much needs to be done in the very near future. TRC's future will address more and more issues that are related to managing co-infections, especially Human Immunodeficiency Virus (HIV) and TB. TRC is on the move.

In the following pages the hard work during the year 2005-06 and the outcome of it is provided. I wish the readers of this annual report to critically review it and provide feedback to us, so that we will make all our efforts to keep doing better and better.

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ABBREVIATIONS

3TC	Lamivudine
AES	Allelic Exchange Substrate
ACR	Alpha Crystalline Protein
ATT	Anti-TB Treatment
ART	Antiretroviral Therapy
ARTI	Acute Respiratory Tract Infection
AZT	Zidovudine
BCG	Bacillus Calmette Guerin
BL	Blood Cells
BMI	Body Mass Index
CBA	Cytometric Bead Array
CFU	Colony Forming Units
CFP	Culture Filtrate Protein
CYP2B6	Cytochrome P450 2B6
DC	Dendritic Cells
DOTS	Directly Observed Treatment Short course
DNA	Deoxyribonucleic Acid
DR	Direct Repeat
d4T	Stavudine
EAI	East African Indian
ECG	Electrocardiogram
EDP	Electronic Data Processing
EFV	Efavirenz
ELISA	Enzyme Linked Immunosorbant Assay
EMB	Ethambutol
FDC	Fixed Dose Combinations
FGDs	Focus Group Discussions
GFATM	Global Fund Against AIDS, Tuberculosis (TB) and Malaria
GFX	Gatifloxacin
GLAS	Graphical Library Automation System
HIV	Human Immuno Deficiency Virus
HLA	Human Leucocyte Antigen
HPLC	High Performance Liquid Chromatography
HHC	Healthy Household Contacts
HIVVT	HIV Vaccine Trial
HR	Heart rate
HRQoL	Health related quality of life
HSV	Herpes Simplex Virus
ICMR	Indian Council of Medical Research
IID	Independent and Identically Distributed
INH	Isoniazid
LIA	Line Immuno Assay
LRP	Luciferase Reporter Phage
OFX	Ofloxacin

MBL	Mannose Binding Lectin
MCMC	Markov Chain Monte Carlo
MDDC	Monocyte-Derived Dendritic Cells
MDR-TB	Multi Drug Resistant TB
MOI	Multiplicity of Infection
MFX	Moxifloxacin
MVA	Modified Vaccinia Ankara
NACO	National AIDS Control Organization
NGO	Non-Governmetnal Organization
NNRTI	Non-NRTI
NRTIs	Nucleoside Reverse Transcriptase Inhibitors
NVP	Nevirapine
PBMC	Peripheral Blood Mononuclear Cells
PC	Personal computers
PCR-RFLP	Polymerase Chain Reaction – Restriction Fragment Length Polymorphism
PDF	Portable Document Format
PF	Pleural Fluid
PFMC	Pleural Fluid Mononuclear Cells
PPs	Private Practitioners
PPD	Purified Protein Derivative
PZA	Pyrazinamide
RNTCP	Revised National TB Program
RMP	Rifampicin
SSC	Short-Course Chemotherapy
SSIG	Semi-Structured Interview Guide
TB	Tuberculosis
TLR	Toll like receptors
TP	Tuberculous Pleuritis
TU	Tuberculosis unit
USAID	United States Agency for International Development
VCO2	Carbon dioxide production
VE	Minute ventilation
VO2 max	Oxygen consumption
WHO	World Health Organization

INTERNATIONAL AIDS VACCINE INITIATIVE

Studies in progress:

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 in HIV-uninfected, healthy volunteers

Background:

In view of the existing burden of Human Immuno Deficiency Virus (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 National AIDS Control Organization (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 as the vector has been initiated.

Aims:

To determine the safety and tolerability of three injections of the vaccine or placebo in two dose levels.

To determine the immunogenicity of this vaccine by measuring HIV-1 specific T- cell responses quantified by ELISPOT INF-γ and serum binding antibody responses to HIV measured by ELISA.

Methods:

A total of 32 volunteers will be enrolled in two groups and followed up for a total of 18 months as per the schedule given below:

Group	Sample size Vaccine/Placebo	Investigational product		
		Month 0	Month 1	Month 6
A	12 /4	IM $5x10^7$ pfu	IM $5x10^7$ pfu	IM $5x10^7$ pfu
B	12 /4	IM $2.5x10^8$ pfu	IM $2.5x10^8$ pfu	IM $2.5x10^8$ pfu

Results:

The trial site was renovated in March 2005 and the staff recruited for the trial has been trained in Good Clinical Practices, Good Clinical Laboratory Practices and gender issues. After the installation of the laboratory equipments, cross validation of all the laboratory procedures was completed by December 2005. Mandatory

approvals from the ethical committees, Drugs Controller General of India, Genetic Engineering Approval Committee and the Health Ministry's screening committee were obtained by end of December 2005. Community advocacy activities for recruitment of volunteers were initiated thereafter in collaboration with YRG Care Centre.

Ten volunteers have been recruited (5 females) by the end of March 2006. A total of 32 volunteers are required to be recruited into the study and the study is expected to be completed by middle of 2008 once all the recruited volunteers complete the twelve month follow up after the last injection.

CLINICAL RESEARCH

Studies Completed:

The long term status of sputum positive pulmonary tuberculosis patients successfully treated with short course chemotherapy

Background:

Information on the long-term clinical, radiological and bacteriological status of patients successfully treated with short course chemotherapy (SCC) regimens — both daily and intermittent — remains largely unknown. However, data on the long term follow up of patients treated with short course chemotherapy would be of great value while analyzing the long term impact of the disease and its treatment.

Aim:

To carry out a one time assessment of the clinical, bacteriological and radiological status including estimation of quality of life and pulmonary function of sputum positive pulmonary TB patients successfully treated with short course chemotherapy regimen at 15-20 years after completion of treatment.

Methodology:

All sputum positive pulmonary TB patients who were started on treatment during the period 1986-1990 at the centre and completed 60 months follow up, formed the study population.

In this prospective study, the patients were subject to the following investigations:

(i) Detailed history regarding re-treatment taken for TB;

(ii) Information collected on smoking, alcoholism, respiratory symptoms and co-morbid conditions like diabetes, hypertension, bronchial asthma, cardiac and renal problems;

(iii) Clinical examination, X-ray chest – PA view, sputum examination by smear and culture for *M. tuberculosis*, urine examination for albumin, sugar, other relevant blood tests, pulmonary function test and ECG. Further, to assess the quality of life the St. Georges Respiratory Questionnaire and the WHO DASII Questionnaire were used.

Results:

Out of the 601 patients treated during 1986-1990, 364 were eligible for the study. This included 163 treated daily and 201 with an intermittent regimen. The mean period of long term follow-up was 16.5 years (range 15-18 yrs). Fig.1 gives the current status of the study population. Sixteen per cent migrated and as for the remaining patients, the coverage was 87 per cent.

Mortality:

Fifty two patients expired (14 per cent). The mean age of these patients was 50 years, and of them, five were females.

Re-treatment:

There were 25 patients (7 per cent) retreated for TB. Out of the 25 patients, 23 were re-treated for pulmonary TB and two for extra pulmonary TB (brain tuberculoma, TB lymphadenitis). The majority of these patients (i.e. 21 of them) were started on re-treatment at the centre after duration of 10 years from the initial treatment for pulmonary TB.

Profile of patients:

The mean age of the 198 patients was 46 years (range 27-73 years). There were 124 (63 per cent) males, among whom 62 (50 per cent) were smokers.

Clinical:

As many as 58 (29 per cent) patients had complaints such as cough or dysnoea, pertaining to the respiratory system. However, clinical signs and respiratory system examination was almost normal in 99 per cent of the patients.

Bacteriology:

Sputum smear negativity was seen in 193 (97 per cent) and culture negativity in 196 (99 per cent). Among the 5 who had positive smears, 3 were culture negative, while 2 of the patients' cultures, grew atypical mycobacteria, *M. kansasii*.

Radiology:

Among the 86 per cent of patients with abnormal chest x-ray, fibrosis alone was present in 62 (36 per cent); calcification alone in 41 (24 per cent). The features of both, fibrosis and calcification, was seen in 59 (35 per cent) of the patients. There were 28 (14 per cent) patients with a normal chest x-ray.

Radiological progress when compared with x-ray taken 15-20 years ago showed that 157 (79 per cent) patients had the same radiological pattern. Five (3 per cent) patients showed an improvement, which was marked by the decrease in the distribution and extent of lung involvement due to the disease. Eleven (6 per cent) patients had fresh lesions and they had respiratory complaints frequently in the past two years. Among them, 8 of them were smear and culture negative, while 3 were smear positive. Of these, 2 had positive cultures of *M. kansasii*. One patient had pericardial effusion.

Electrocardiogram (ECG):

ECG findings were normal in 160 (81 per cent) of the patients. Features suggestive of corpulmonale were observed in 21 (11 per cent) of the patients, with predominant findings of right arterial overload and right ventricular enlargement.

Spirometry:

Pulmonary function tests were performed in 148 patients, which showed that the lung function was normal in 57 (39 per cent) of them, while 47 (32 per cent) and 10 (7 per cent) showed restrictive and obstructive type of disease respectively. Combined (both restrictive and obstructive) type of disease pattern was observed in 34 (23 per cent).

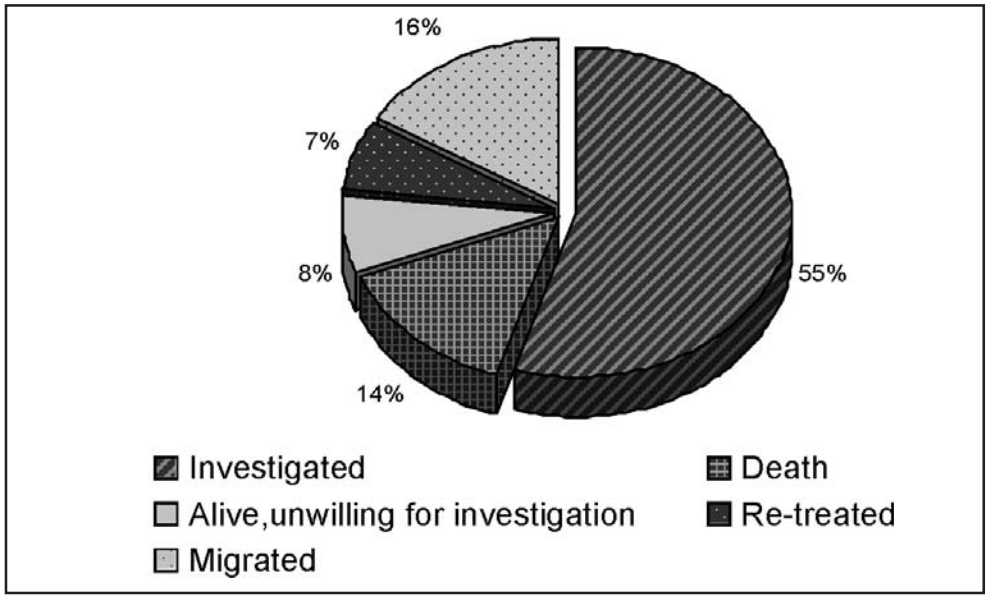
Males had significant lung function impairment as compared to females ($p<0.001$). Among the males, lung function was interpreted as normal in 41 per cent, restrictive type in 20 per cent, obstructive type in 10 per cent, and combined type in 29 per cent. Lung function impairments were similar when it came to smokers and non-smokers.

Among the females, 34 per cent, 54 per cent and 12 per cent had normal lung function, restrictive and combined disease patterns respectively. None of them had an obstructive type of disease. Yet restrictive type of disease was observed more in females ($p=0.01$). The data collected on the quality of life of these patients, is being analyzed.

Conclusion:

The study has shown that it is possible to get information on 84 per cent of the TB patients treated 15 to 20 years earlier. We observed that 71 per cent had no respiratory complaints and all investigated patients were culture negative for *M. tuberculosis.* Fourteen per cent had normal chest x-ray, while the remaining had residual lesions. The ECG evidence of corpulmonale was present in 11 per cent. The lung function was normal in 39 per cent.

Fig.1: Current status of the cohort of pulmonary TB patients treated with short course chemotherapy 15 – 20 yrs ago



Studies in progress:

Study of the efficacy and tolerability of moxifloxacin and gatifloxacin containing regimens in the treatment of patients with sputum positive pulmonary TB

Background:

A randomized clinical trial carried out by TRC has demonstrated that patients with sputum-positive pulmonary TB, can successfully be treated with a four-month regimen that substituted ofloxacin (OFX) for ethambutol (EMB) in the four-drug intensive phase given daily (IJT 2002).

However, a similar regimen administered thrice weekly was less successful (TRC Annual Report 2003-04). Meanwhile, the newer generation of fluoroquinolones, moxifloxacin (MFX) and gatifloxacin (GFX), have shown to have more potent anti-mycobacterium activity compared to OFX. Therefore it was decided to study the efficacy and safety of thrice-weekly MFX and GFX regimens in the treatment of patients with smear-positive pulmonary TB. This was done in a randomized clinical trial.

Methods:

Newly diagnosed sputum smear-positive pulmonary TB patients residing in or around Chennai and Madurai, who fulfill the inclusion criteria are randomly allocated to one of the following three treatment regimens:

Regimen 1: $2GHRZ_3$ / $2GHR_3$. GFX, isoniazid (INH) and rifampicin (RMP) thrice weekly for four months with pyrazinamide (PZA) for the first two months.

Regimen 2: $2MHRZ_3$ / $2MHR_3$. MFX, INH and RMP thrice weekly for four months with PZA for the first two months.

Regimen 3: $2EHRZ_3/4HR_3$ as a control regimen. INH and RMP thrice weekly for six months with EMB and PZA for the first two months.

It is proposed to admit 300 patients to each arm. The study is being conducted in Chennai and Madurai.

Results:

The study was started in May 2004 and 391 patients have been enrolled till April 2006. This includes 141 patients in regimen 1, 92 in regimen 2 and 158 in control regimen (Regimen 3). The study is ongoing.

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

The immunomodulator containing *Mycobacterium w* was developed by the National Institute of Immunology, New Delhi in 1980. It has been found to be useful in the prevention of TB in experimental animals. A pilot study conducted to evaluate the role of *Mycobacterium w* in improving sputum conversion rate in pulmonary TB, showed that the conversion rate was faster when *Mycobacterium w* was added to the short course chemotherapy. Immunomodulators work against persistors, which may result in reducing the relapse rates. The addition of immunomodulator to chemotherapy is well tolerated and does not increase the adverse reactions to the therapy.

A double blinded, randomized, placebo controlled, multicentric clinical trial has been initiated by the Department of Science and Technology. This is being done with the object of studying the cure rate in Category II pulmonary TB patients after the addition of the *Mycobacterium w* vaccination to standard anti-TB drugs. The patients are randomly chosen to receive either the vaccine or placebo along with the standard category II RNTCP regimen. One hundred and twenty eight patients are proposed to be admitted to the trial. The study has begun in March 2006, and so far 3 patients have been enrolled.

Evaluation of chemotherapy regimens for TB in HIV infected persons (Funded by ICMR Task Force on HIV-TB)

Background:

The duration of anti-TB treatment among HIV positive patients with TB, is still a contentious issue. A six-month intermittent (3 times a week) regimen is the standard treatment for TB in RNTCP in India and many countries.

Aim:

1. To evaluate the efficacy of RNTCP treatment regimens among HIV patients infected with TB
2. To compare the efficacy of a six-month versus a nine-month intermittent anti-TB regimen among HIV positive patients with TB

Methodology:

This is an ongoing, prospective, randomized, controlled clinical trial.

Arm A: Six-month regimen $2EHRZ_3/4RH_3$.

Arm B: Nine-month regimen $2EHRZ_3/7RH_3$

The drug dosages are EMB: 1200 mg, INH: 600 mg, RMP: 450mg in patients with <60kg/600 mg >60 kg and PZA:1500 mg with Pyridoxine 10 mg), given thrice weekly.

All those HIV positive patients diagnosed with TB, based on sputum smear and culture or radiologically suggestive of TB, were included in the study. Randomization was done by a permuted block scheme and stratified by a CD4 cell count (<200 & ≥200 cells / cu.mm), and smear grading (0, 1+ & 2+, 3+). Treatment was fully supervised for the first two months, then brought down to once a week. The intensive phase was extended by four weeks if sputum smears were positive at the end of second month. Follow up of patients was done every month with clinical examination, sputum AFB smear and culture for *M. tuberculosis*. Chest radiograph and CD4 counts were done at baseline, during the second month and at the end of the therapy. None of the patients were on antiretroviral therapy (ART) at the time of intake. End points of the study are sputum culture negativity at the end of treatment and relapses during follow-up. Intent to treat analysis and on treatment analysis, will be performed.

Results:

Up to March 2006, 382 patients were admitted to the study. Forty two patients were treated with the RNTCP Cat II regimen and separately analyzed. Six patients were initially excluded. Out of the 334 patients, 230 had pulmonary TB confirmed by sputum culture. Fifteen patients were excluded for treatment analysis (13 had less than 80 per cent of drug dosage and there were two early deaths).

Among the 215 patients included in the analysis, 110 were allocated to regimen A (six months) and 105 were allocated to regimen B (nine months). The baseline demographic and other characteristics of the patients admitted to the two treatment arms are given in Table 1.

Seventy seven per cent of the patients had smear positivity. The median CD4 cell counts were 132 and 167 cells / cu.mm in both the regimens. The mean age of the patients was 35 years. The CD4 count was under 200 cells / cu.mm in 66 per cent of the patients suggesting severe immunosupression. At the end of the intensive phase, sputum smear conversion rate was 61 per cent while culture conversion was 87 per cent. Among patients available at the end of treatment (n=172), the culture negativity at the end of treatment was 98 per cent and 95 per cent in the two regimens respectively (Fig.2). Overall, there was an 83 per cent favourable response in regimen A and 76 per cent in regimen B (Table 2). Change of treatment due to bacteriologic or clinical deterioration (failure) and deaths in the two arms are shown.

Drug susceptibility pattern of the organisms isolated from pre treatment sputum culture, showed that 86 per cent of the organisms isolated were susceptible to all anti-TB drugs, and that resistance to both INH and RMP (MDR-TB) was seen in 2 per cent of the patients. Occurrence of adverse reactions was similar in both groups. A follow up is ongoing to study the relapse rates.

Table 1: Baseline characteristics (n=215)

	Regimen A 6m (n=110)	Regimen B 9m (n=105)
Males %	84.5	74.3
Mean Age (Years)	34.1	34.8
Mean Weight (kg)	43.4	43.1
Sputum smear positivity %	76	78

Fig.2: Smear and Culture Results at the end of treatment (n=172)

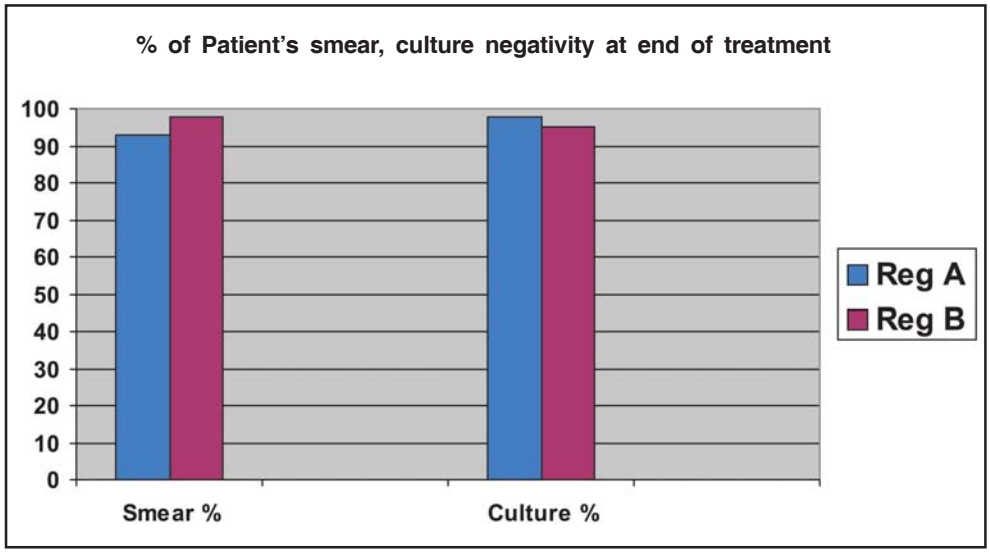


Table 2: Outcome on treatment

Outcome		Regimen A (n=109)	Regimen B (n=102)
Favourable		91 (83%)	78 (76%)
	Rx changed	13 + 1 (Toxicity)	14
Unfavourable	Total deaths (with active TB)	4 (1)	10 (3)

Preventive Therapy for TB in HIV-infected persons (Funded by USAID)

Background:

Persons co-infected with *M. tuberculosis* and HIV, have a 5-8 per cent annual risk and a 50 per cent or greater lifetime risk of developing active TB.

The increased risk of developing TB among the HIV-infected prompted a need to consider institution of preventive measures, so that HIV positive patients are enabled to avoid the risk of progression to clinical TB.

Aims:

The study is being done to compare the efficacy of 2 regimens (INH 300 mg daily for 3 years versus EMB 800 mg with INH 300 mg daily for 6 months) in reducing the incidence of TB and mortality among HIV-infected persons.

Methods:

HIV positive persons greater than 15 years without evidence of active TB and meeting eligibility criteria were randomly selected to receive either of the 2 regimens (INH 300 mg daily for three years or EMB 800 mg with INH 300 mg daily for 6 months, stratification based on tuberculin test reaction of 5mm). Patients collected the drugs for self-administration once in 15 days and surprise home visits were done to check the pill count and also collect urine for acetyl INH measurements. All patients were given Pyridoxine 10 mg daily. Clinical examination was done every 3 months, while complete investigations including chest X-ray, sputum examination and CD4, CD8 counts done every 6 months, and at any time if clinical deterioration was present. Follow up is for 3 years and end points are development of TB and death.

Results:

Seven hundred and eleven patients have been admitted to the study up to March 2006. Seventy nine were excluded for various reasons (pre treatment culture positive-29, treatment less than 80 per cent - 41, treatment changed within 1 month – 8, early deaths within 1 month – 1). Out of the 632 patients in analysis, 317 were allocated to 6 months of EMB-INH daily and 315 to 36 months of INH daily.

Table 3 shows the baseline characteristics of patients admitted to both arms. Among patients who had completed 36 months, (as on March 31, 2006), 108 in EH regimen and 103 in INH regimen, TB developed in 16 and 12 patients respectively. The follow-up is in progress.

Table 3: Baseline characteristics

	6EH (n=317)		36H (n=315)	
	Mean ± S.D.	Range	Mean ± S.D.	Range
Age (Years)	29.8 ± 7.2	18 - 57	30.3 ± 7.0	18 - 60
Weight (kgs)	50.7 ± 9.8	30 - 79	49.3 ± 10.2	30 - 97
Mx-mm	7.8 ± 9.6	0 – 40	7.4 ± 9.3	0 - 35
	Median	25-75 Percentile	Median	25-75 Percentile
CD4	337	208-529	330	194-475

Exercise limitation in patients treated for Pulmonary TB

Background:

The long-term functional sequelae of pulmonary TB are not well described. Pulmonary TB is a wasting disease, which also affects skeletal muscle mass, limiting manual work capacity.

Aim:

To measure maximal work capacity and cardio respiratory functions in patients treated for smear positive pulmonary TB with standard short course chemotherapeutic regimens. This data would be compared to that obtained from healthy age and sex–matched controls.

Methods:

Oxygen consumption (VO2 max), carbon dioxide production (VCO2), minute ventilation (VE), heart rate (HR) and workload were measured with a progressive incremental exercise test using the Jaeger oxycon-pro metabolic cart and a treadmill. Oxygen saturation was monitored with a pulse oxymeter. Sixty four patients who had completed a course of anti-TB therapy and 72 sex, age and height matched controls from the same ethnic group were studied.

Result:

The mean height of patients and controls was similar. But weight ($p < 0.02$) and BMI ($P < 0.01$) were significantly lower among TB patients. The metabolic and ventilatory parameters at peak exercise in patients and controls are shown in Table 4. Male TB patients had significantly lower aerobic capacity and minute ventilation at maximal exercise and achieved lower peak heart rate. When corrected for body weight, the maximal oxygen consumption was similar. The peak heart rate achieved was lower among patients, though all achieved an RER > 1, indicating they had exercised maximally. Further, 20 per cent of patients and three per cent controls, had desaturation at maximal exercise.

Conclusion:

Patients treated for pulmonary TB have exercise limitation as demonstrated by lower peak heart rate, VO2 max and maximal ventilation. Males are affected more than females. A significant proportion of patients desaturated at maximum exercise. These findings suggest that the work capacity of patients treated for pulmonary TB is significantly impaired and this could affect their quality of life.

Table 4: Cardio-respiratory parameters at maximal exercise in controls and TB related patients

	PATIENTS				CONTROLS			
	Females (n=29)		Males (n=35)		Females (n=35)		Males (n=69)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Load (Watts)	123**	54.7	204**	103.1	196	57.0	311	81.2
VO2 (ml/min)	1237	326.1	1872**	562.8	1410	389.2	2449	591.5
VCO2 (ml/min)	1345**	397.7	2066**	788.7	1678	508.0	2923	782.7
VE (L/min)	40*	11.3	60**	24.1	46	11.7	79	22.7
HR (/min)	158	25.8	160	35.9	171	16.0	171	32.4
RER	1.089**	0.15	1.0834**	0.18	1.186	0.133	1.191	0.12
VE/VO2	31		30		31		32	
VE/VCO2	28.*		28		27		27	
ETO2 (%)	16.07*	0.8	15.65*	1.1	16.2	0.8	16.1	0.9
ETCO2 (%)	5.19*	0.67	5.6	0.95	5.4	0.93	5.5	0.74
**P<0.01	compared to controls							
*P<0.05								

Nutritional assessment and supplementation in HIV-infected patients with and without TB (Funded by World Food Programme, New Delhi)

Background:

Tuberculosis and HIV infection are known to be separately associated with malnutrition. TB might worsen the course of HIV associated immunosuppression and therefore reduce the rate of survival among HIV-infected subjects. Despite the high prevalence of TB and malnutrition among HIV seropositive patients, data concerning the nutritional status of TB/HIV co infected patients in developing countries like India, is scarce.

Aims:

1. To document the occurrence of baseline macro and micronutrient deficiencies in HIV infected individuals in south India and correlate that with their immune status.
2. To test the efficacy of an intervention in the form of a nutritional supplement and quantitate changes in nutritional, biochemical and immunological parameters over a period of 6 months.

Methods:

The study was started in July 2003. HIV infected persons without TB and those who had completed therapy for TB, formed the study population. A baseline clinical, anthropometric and dietary assessment along with laboratory investigations (hematology, biochemistry and immunology) was done for all patients at the time of enrollment to the study.

Patients were divided into two groups: The first group, the intervention group, received a high calorie, high protein supplement called "Indiamix" supplied by the World Food Programme, New Delhi. Each patient had to consume 100gms per day, which supplies an additional 400 calories and 15gms of protein. The other group, the control group, did not receive the nutritional supplement for the first six months. Patients were followed up clinically (including dietary assessment and anthropometric measurement) every three months and hematological, biochemical and immunological investigations, were repeated every six months. Body composition was measured by BIA and fat content and fat-free mass calculated. The study has been completed and data analysis is ongoing.

Interim results:

Six hundred and sixty two patients were enrolled into the study. Of these, 551 patients received the supplement (cases) and 111 did not receive any supplement for a period of 6 months. The latter group served as controls for the study. At the end of a 6-month follow up, data is available for 288 patients, that is, 83 controls and 214 cases. Baseline characteristics like age, sex, socioeconomic status, body weight and BMI, were comparable between the two groups. Ninety per cent of the patients were from the lower socio-economic strata with the mean age being 31.5 $\pm$ 6 years. Table 5 shows the changes in various parameters after 6 months of nutritional supplementation.

Conclusion:

After 12 months of nutritional supplementation, there was a significant increase in weight, BMI, mid arm circumference, hemoglobin and CD4 cell count in the supplemented group compared to controls. Maximum improvement was observed in the lowest CD4 cell strata. The study findings suggest that nutritional supplementation can help to improve nutritional status and delay disease progression in HIV positive persons.

Table 5: Changes in nutritional parameters after 6 months of supplementation

Subjects (n=214)	**BL**	**12 months**	**p value**
Mean $\pm$ SD			
Weight	49.9 $\pm$ 9.73	51.2 $\pm$ 10.35	<0.001
BMI	20.6 $\pm$ 3.59	21.1. $\pm$ 3.75	<0.001
MAC	23.9 $\pm$ 3.28	24.7 $\pm$ 3.71	<0.001
n=195			
Hb	11.6 $\pm$ 1.69	12.2 $\pm$ 1.68	0.001
CD4	376 $\pm$ 234	422 $\pm$ 251	0.001
Controls (n=78)	**BL**	**6 months**	**p value**
Weight	51.9 $\pm$ 12.88	52.0 $\pm$ 12.17	NS
BMI	21.28 $\pm$ 4.77	21.35 $\pm$ 4.39	NS
MAC	24.1 $\pm$ 4.39	24.4 $\pm$ 4.58	NS
Hemoglobin	12.1 $\pm$ 1.69	12.1 $\pm$ 1.57	NS
CD4 cell counts	448 $\pm$ 278	396 $\pm$ 286	0.033

MAC - Mid arm circumference

SOCIOLOGICAL RESEARCH

Studies Completed:

Knowledge and attitude on HIV vaccine trials and willingness to participate among persons at risk in south India

Background:

The TB Research Centre is conducting a phase-I HIV Vaccine Trial (HIVVT). Prior to any HIV preventive vaccine trial, it is important to assess the concerns, knowledge gaps, attitude towards HIV vaccines and willingness to participate in future HIVVTs among populations at risk of HIV infection. The triangulation of qualitative and quantitative data, will give meaningful information about HIVVT participation. To address these issues, a sociological study with a two-phase qualitative-quantitative approach, was conducted. This was performed to find out the HIV vaccine readiness at Chennai and Madurai. The study was done in collaboration with the University of California, Los Angeles, USA.

Objectives:

1. To determine the willingness of populations at risk of HIV infection to participate in future HIVVTs.
2. To evaluate the knowledge levels of vaccine trial concepts.

Methods:

The participants were 112 men and women representing the following subgroups:

1. Transport workers, such as truckers and cleaners;
2. Clients who attended a STD clinic at Government General Hospital in the last three months;
3. Injection drug users;
4. Men having sex with men;
5. Women in sex work.

In addition, representative samples of monogamous married women from the self-help groups in the local communities were included. The study participants were mainly drawn from four major non-governmental organizations (NGOs) - both at Chennai and Madurai. A Community Advisory Board assisted in the design of the semi-structured Interview Guide that guided the focus group questions and the revision of measurement scales. For Phase II, interview schedules along with modified measurement scales were administered to 501 study participants, 83 approximately from each group.

Findings of Phase – I (Qualitative Research)

A total of 12 Focus Group Discussions (FGDs) were conducted with six – seven eligible participants per group. Two focus groups were conducted with each subgroup resulting in a sample size of 112. During the pilot study, researchers came to know that the knowledge on HIVVTs was very minimal among the study participants. Therefore they were educated about HIVVTs, particularly about the double-blind selection into the vaccine or placebo groups, the possibility of vaccine induced sero-positivity, the partial efficacy of experimental vaccines and the need to practise safer sex. A Semi-Structured Interview Guide (SSIG) guided each

session using open – ended questions. All FGDs were audio taped, transcribed and translated into computer files. Content analysis was performed using the constant comparative method (Glaser, 1978).

Participants expressed keen interest in future HIVVTs. Factors facilitating willingness to participate in future trials included altruism and the desire to have a protective vaccine for the future. Despite expression of trust in the Government, participants still felt that specific requirements would be necessary to ensure adequate recruitment of participants. The requirements included, assurances regarding stigma and confidentiality, compensation for families in the event of a poor outcome with a future HIVVT. Additional concerns centered on the impact of the vaccine on the recipient's physical health and the implications of seroconverting. Participants were worried about the possibility of risk behaviors increasing after the receipt of vaccination.

The critical need for ongoing education and counseling to make people aware of the dangers of engaging in risky behavior during and after participating in a future HIVVT has been discussed, and implications for mass media have been suggested.

Findings of Phase II (Quantitative Research):

Out of the 501 respondents, 55 per cent were males, 59 per cent from the age group of 31–50 years; 64 per cent married; 79 per cent school educated and 73 per cent working. The participants' baseline knowledge on HIVVTs was assessed before educating them about the vaccine concepts. The same scale was administered again to assess their level of understanding. The difference was statistically significant for all 10 items in the Koblin scale and the attitude about the HIV vaccine was assessed by an 18-item CDC Vaccine Attitude Scale, which showed how much they agreed or disagreed with each statement.

Attitudes about the HIV vaccine was assessed by an 18-item HIV Vaccine Attitude Scale, and subscale clustering included benefits of participation, concerns and barriers for participation and future sexual behavior change after vaccination. More than 70 per cent agreed that the HIV vaccine would protect them from HIV infection and hoped that HIV could become preventable like polio.

About 51 per cent worried about the effects of a HIV vaccine on their lives and 46 per cent were concerned about whether ''the vaccine was powerful enough to prevent the HIV infection.'' Additional concerns were the restriction on travel due to participation (39 per cent) and the impact of the vaccine on the participants' ability to get married, insurance and job prospects (38 per cent). Less than 30 per cent of them worried about whether they would be given the vaccine or placebo. Overall, 76 per cent of the respondents agreed that sex without a condom is not safe, whether there was a HIV vaccine available or not. Overall, willingness to participate for HIVVTs was 82 per cent. The main reason was protection from HIV infection and altruism. Many participants said that their participation in a HIVVT was important for the common good of India. Besides, the research might help in the eradication of the HIV infection.

Women participants expressed their desire to protect themselves from infected husbands. The reasons for refusal to participate were perceptions about not being at risk of the HIV infection; fear of stigma; uncertainty about the receipt of vaccine or placebo; concern about the safety of the vaccine.

Difference in the level of knowledge (% correct responses) at base-line and after vaccine education is shown in Table 6. About 57 per cent wanted to seek advice

from others before giving their consent for participation and the majority of them wanted to consult their respective NGOs. It is interesting that the pattern of willingness is different when we assess the association between the participants' demographic variables and the willingness to participate in HIVVTs. Willingness was 84 per cent among the males and 81 per cent among females. It was more among those who had never married, separated, widowed than those married (89 per cent, 96 per cent, 100 per cent vs. 77 per cent), not educated than the highly educated (63 per cent vs. 94 per cent), and those employed than the unemployed (84 per cent vs. 79 per cent).

It is likely that high-risk volunteers will be willing to enroll in HIVVTs. Barriers and concerns should be dealt with carefully by providing correct information. The local NGOs play a crucial role in motivating trial volunteers.

Verbatims during FGDs:

"HIV/AIDS is a killer disease. It would be wonderful if there is going to be a vaccine for it. We are confident that it would come" (MSM)

"Developing a vaccine itself is wrong" (TW)

"For people who live by Indian culture standards, it is difficult to enroll them" (TW)

"Other people may get to know about the result. No one will come forward due to stigma attached to HIV/AIDS"

"Following vaccination, is it safe to have sex with wife? Friends? Will they show positive result?"(MSM)

"Among the sexual partners, if one is vaccinated and the other one is not vaccinated, will they get the infection due to sexual relationship?"(CSW)

"People will solicit improper relationships with gusto" (MW)

"There is a fear that AIDS is a deadly disease and death is certain. This fear will disappear in the future" (TW)

"We cannot tell our regular customers to use condoms nor can we be without job" (CSW)

"No one would want to catch the disease-most would use the opportunity & reform themselves" (MSM)

Table 6: Difference in the level of knowledge (% correct responses) at base-line and after vaccine education

Items	Pre-test (%)	Post-test* (%)
Vaccine safety	86	93
Strengthens immunity	52	89
Enrolls both HIV+ and HIV-	46	77
Healthcare for volunteers	70	84
Be informed about Vaccine/Placebo	38	75
Guarantee for future HIVVTs	36	85
No effect on HIV test results	47	68
Get vaccine or placebo	32	90
Not 100% effective	26	63
Study nurse will select	37	90
No. of responses = 501		

* There is a statistical difference between the pre and post-test at 95% confidence interval for all items

Epidemiological Research

Studies in progress:

Mortality among a cohort of TB patients treated under RNTCP from a rural area in India

Objectives:

1. To measure the mortality rates among the cohorts of TB patients in a rural area such as Tiruvallur;
2. To measure the excess general mortality among the cohorts of TB patients;
3. To identify high-risk groups for mortality among TB patients;
4. To study the trend of mortality rates over successive annual cohorts of TB patients;

Methods:

Study design:

This is a retrospective cohort study.

Study population:

The study population consisted of 3429 patients (2469 males and 960 females) registered with Velliyur TB Unit (TU), Tiruvallur district, Tamil Nadu, in the years 2000, 2001, 2002 and 2003. They were treated under the RNTCP and were all retrospectively followed-up from the day of the start of treatment to either the date of interview (for the survivors), or the date of death (for the dead).

There were 2729 (79.6 per cent) survivors and 700 (20.4 per cent) deaths.

Study duration:

May 2005 to February 2006.

Data analysis is in progress.

Mortality surveys in Andhra Pradesh and Orissa

This is a Government of India Project (Central TB Division) funded by Global Fund Against AIDS, TB and Malaria (GFATM). The total budget sanctioned for this project is Rs. 85 lakhs.

Objectives:

1. To estimate crude mortality rate in the state of Andhra Pradesh.
2. To estimate TB mortality rate among the general population aged $\geq$ 15 years.

Sample Size:

380,000 for both the States

Sampling design:

The sampling frame consisting of a list of villages (rural) and towns (urban) were obtained from the Directorate of Census Operations. In the rural areas, villages were the sampling units. In the urban areas, sampling units were from the census enumeration block. The total sample size of 380,000 will be selected from 380 sampling units. All these sampling units have been selected by a simple random method without replacement.

Methodology:

Health workers registered the study population by house-to-house enumeration. During registration, the household number, names of the members, age in completed years and gender were recorded in the household form. In addition, information on occurrence of death in each household was recorded. All household forms that report deaths will be handed over to the supervisors for detailed verbal autopsy to ascertain the cause of death.

Verbal Autopsy:

All deaths identified during enumeration were probed in detail by the supervisors through verbal autopsy from a close relative / neighbour / friend to ascertain the cause of death. Supervisors are specially trained to undertake verbal autopsy. Verbal autopsy is an investigation of train of events, circumstances, symptoms and signs of illness leading to death through interviews of relatives or associates of the deceased.

Andhra Pradesh:

The survey began during the first week of September, 2005.

Surveys in five districts, Mahabub Nagar, Khammam, Krishna, Vizianagaram and Prakasam were completed. The survey is in progress in Chittoor district.

Cumulative coverage: Units-370; Population-3,76,742; Deaths-2114.

Orissa:

The survey began in the last week of October 2005.

Surveys in six districts namely, Cuttack, Jagathsinghpur, Kendra Para, Sundargarh, Deogarh and Gajapati have been completed. Survey is in progress in Bargarh and Rayagada districts.

Cumulative coverage: Units-330; Population-3,34,417; Deaths-1612.

Model DOTS Project:

Directly Observed Treatment Short course (DOTS), a global strategy for control of TB is being implemented in India in a phased manner since 1997. The epidemiological impact of this strategy in high burden countries is not known. To understand this, the TB Research Centre, is undertaking an epidemiological impact study in five blocks of Tiruvallur district, Tamil Nadu. This is the same area where the BCG trial was done. Therefore, epidemiological data on TB is available even before DOTS has been implemented. The project has its technical support from the World Health Organization (WHO) and financial support from United States Agency for International Development (USAID). Started in May 1999, the project has the involvement of TRC in the following areas:

a) Training

b) Epidemiological survey of TB disease and infection

c) Bacteriological and molecular epidemiological studies and

d) Operational research.

Training:

Good quality training is essential for the successful implementation of any programme. Therefore TRC has been identified as a nodal centre for training in RNTCP. During the training period, 53 Medical Officers (Tr), 41 Medical Officers, 59 Senior Treatment Supervisors, 19 Senior TB Laboratory Supervisors, 13 Laboratory Technicians and nine microbiologists, have been trained.

Studies completed:

Assessment of quality of life of TB patients treated under the DOTS programme

Background:

Information on health related quality of life (HRQoL) of treated TB patients is very sparse.

Objective:

To assess the HRQoL of TB patients, one year after treatment completion.

Method:

Patients registered under the programme (July 2002-June 2003) in one tuberculosis unit (TU), South India, one year after the successful completion of treatment were interviewed. Data on HRQoL was collected using the modified SF-36 questionnaire, covering physical, mental, social and economic well being. Scores were given for all domains and the total well being score was the average of all these domains.

Results:

Of the 436 TB patients interviewed, overall well being score was 72, on a scale of 100. The mean scores for different domains were: social (84), physical (74), mental (68) and economic (62). The total well being score was significantly less (70) for patients aged $\geq$45 years and for non literate patients (68). Persistence of symptoms was observed among 40 per cent of the patients. The total score was (65) in this group was significantly lower compared to persons without symptoms (77).

Conclusion:

The HRQoL was significantly impaired among those with persistent symptoms. This information is vital for developing the targeted communication strategies.

Smear-positive TB patients at 2-3 years after initiation of treatment under a DOTS programme in a district, south India

Background:

The DOTS strategy aimed at least 85 per cent cure rate and a case detection of at least 70 per cent. This reduces chances of failure, relapses and prevents the harbouring of Multi Drug Resistant TB (MDR-TB).

TRC monitored the programme in one TU in Tiruvallur district intensively, for 5 years since its implementation in 1999. This was done by using different operational studies and generated valuable data. But the status report of cases treated under a DOTS programme is yet to be looked into. This information would show ways and means of reducing mortality and morbidity.

Objective:

To investigate how cases 2-3 years after the initiation of treatment under DOTS in the same area are faring.

Methodology:

Smear-positive TB patients registered for treatment during the year 2002 and 2003 formed the study population. Those who were declared successfully treated, defaulted or failed were under a follow-up at 3 and 2 years respectively, after the initiation of the treatment. Two sputum specimens were collected from those who were available at the time of the visit by the health worker at their residence.

Results:

A total of 1171 smear positive cases were registered for treatment during the period 2002-2003. Of these, 1113 cases were eligible for follow up and among the 1088 cases that were followed up, 148 expired, 54 migrated and 46 cases could not be contacted. Sputum sample was collected from the remaining 840 cases. The overall mortality rate was 15 per cent and among the remaining, 18.6 per cent (156 of 840) remained positive.

Conclusion:

The mortality and those who had active TB was higher among smear positive cases, followed up at 2-3 years after the initiation of treatment.

Studies in progress:

Epidemiological Impact Study: Community survey of TB infection and disease

Background:

DOTS programme was implemented in Tiruvallur district, Tamil Nadu in May 1999. To assess the epidemiological impact of the DOTS strategy, the TB Research Centre is carrying out a series of sample surveys to estimate the prevalence of disease and infection in this district. The study covers a population of 5, 80,000.

Aims:

To study the trends over time for both disease and infection and thereby, to measure the impact of DOTS implementation.

Methods:

All adults $\geq$ 15 years have been included. They have been screened by two methods, namely, elicitation of symptoms and X-ray examination. Two samples of sputum specimen were collected from those who were either symptomatics and/or X-ray abnormal suggestive of TB. These specimens were processed for smear and culture and those who became bacteriologically positive, were referred for anti-TB treatment provided they satisfied the RNTCP guidelines.

All children included in the tuberculin survey, were tuberculin tested with purified protein derivative (PPD) 1TU RT23 vials. The reaction sizes were read after 72-96 hours.

Results:

Two disease surveys, each with two and half year duration, were completed. The second resurvey is in the completion stage.

Coverage in the current survey is above 90 per cent for all investigations – namely symptoms, X-ray and sputum examination as seen in Table 7. So far, 284 persons have been diagnosed with smear/culture positive TB.

Table 7: Coverage of various investigations

Activities	2nd resurvey
Eligible for symptom and X-ray	96075
Symptom screening	88341 (92%)
X-rayed	87211 (91%)
Sputum eligible	11175
Sputum collected	10514 (94%)

The prevalence of the disease from the two completed surveys, have shown a decline in TB prevalence. This demonstrates the effectiveness of the DOTS implementation, and a more rapid reduction in the prevalence of disease compared to that during the pre-DOTS period. (A precise estimate of the decline will be available only after an analysis and after the completion of the second resurvey).

Based on the existing data generated from Tiruvallur and elsewhere, the burden of TB in India for the year 2000 was estimated at 8.5 million (95 per cent C.I: 6.3-10.4) of which 3.8 million were bacillary cases, 3.9 million abacillary cases and 0.8 million extra-pulmonary cases.

In the third tuberculin survey among 27,199 children eligible for tuberculin testing, 25,935 (95 per cent) were test read.

From among 8329 unvaccinated children, 499 (6.0 per cent) were found to be infected using the cut-off at 12 mm. The acute respiratory tract infection (ARTI) was estimated to be 1.2 per cent. The ARTI estimates in the two tuberculin surveys conducted earlier are 1.6 per cent and 1.4 per cent respectively. There was a significant decline in the trend of TB infection ($P<0.001$). The annual decline estimated from the first to the third survey is six per cent.

Conclusion:

DOTS implementation is associated with a substantial reduction in the prevalence and risk of TB infection amongst children.

Risk of TB infection and disease in different economic strata

Background:

Tuberculosis affects the poorest people in the world. Ninety five per cent of the new TB cases every year are in developing countries. Most researchers agree on the general association between TB and socio economic conditions, but no direct cause and effect relationship has been demonstrated.

Aim:

To estimate TB infection and disease rates in the community and relate these to the economic status of the population.

Methods:

The study is being carried out amongst the same population where the disease survey has been undertaken. This is to evaluate the prevalence of the disease. All households in a village included for the survey, will be visited. The head of the family/informant will be identified and the purpose of the survey will be explained to him/her. Their cooperation therefore is being requested for the interview using the semi-structured questionnaire. This will also reveal their socio-economic status (standard of living index). Data collection started in February 2004. So far, 28,702 households have been interviewed and coverage for the interview has been around 95 per cent. The study is going on.

Reliability of involving community Volunteers as DOT Providers

Background:

DOTS, the main strategy of RNTCP, are the best method currently available to control TB. After seeing the problems being faced by the Government, the role of the DOT providers has been recognized. Therefore decentralizing TB control measures beyond health facility by using Community Volunteers to act as DOT providers to facilitate administration of regular and complete treatment, has been initiated. Identifying a good DOT provider is a challenge. It was proposed to visit and interview all the DOT providers including community volunteers identified as DOT providers and the patients treated by them currently, to obtain detailed information on their practices.

Aim:

1. To assess the reliability and accountability of Government health workers and community volunteers as DOT providers.
2. Acceptability of community providers by patients.

Methods:

Community volunteers engaged as DOT providers during two cohort periods in one TU at Tiruvallur district and those at the Chennai Corporation formed the study population. DOT providers and their patients are interviewed by trained field workers using semi structured questionnaires. These aid in getting information about their practices and problems encountered while acting as DOT provider.

In the rural area, data collection was completed for 201 providers and 377 patients while in the urban area, it has been completed for 52 providers and 55 patients.

Factors that lead to hospitalization of TB patients

Background:

The RNTCP endorses domiciliary treatment under the direct observation of health care staff. Even though hospitalization is recommended in special cases, a large number of beds in the various hospitals throughout the country are still utilized by

TB patients. Though the DOTS strategy is considered a cost effective method of intervention, large numbers of patients still seek inpatient care. As a result of this, considerable resources are spent on care and attention for these patients.

Aim:

To see why there is hospitalization of TB patients in the RNTCP implemented areas.

Methods:

As many as 450 hospitalized TB patients were proposed to be included in the study from select hospitals (TB sanatorium at Tambaram, TB hospital at Otteri, Chennai and TB sanatorium at Madurai).

A pilot study was initiated in February 2005 and based on this the questionnaire was modified. So far, 117 patients have been interviewed. The study is in progress.

Private practitioners and TB: Patient perceptions

Background:

In the pre RNTCP era, it was found that patients who started anti-TB treatment (ATT) with Private Practitioners (PPs), switched over to the public sector. This is because there have been many financial problems. After implementation of the RNTCP, perceptions on TB treatment with PPs, their awareness of DOT, and reasons for choice of treatment are not known. Therefore a study on the patient's perceptions in TB treatment would come in handy.

Aims:

To discover:

1. The proportion of patients treated by the PPs in the study population
2. Reasons for switching over to public sector after being treated by PP
3. The perceptions of patients on PPs

Methods:

The study is being conducted among patients attending urban (Choolaimedu TU, Chennai), rural (Tiruvallur TU), specialized TB hospital (Tambaram TB sanatorium) and in Kancheepuram district.

All patients are interviewed using a semi-structured pre-coded interview schedule. So far, 442 patients from rural, 426 from urban, 281 from TB sanatorium and 102 from Kancheepuram have been interviewed.

Evaluation of the impact of RNTCP on the socio-economic status of TB patients and their families

Background:

An earlier study from TRC, before the implementation of DOTS, has reported that the direct and indirect costs of the TB epidemic come to at least $3 billion (Rs 13,000 crores) every year. Patients suffering from TB incurred a total loss of $99 (Rs.3469) while shopping for diagnosis and treatment. Indian workers with TB lost an average of 83 workdays. This study is aimed at estimating the long term economic impact of the TB control programme after 5 years of the implementation of the DOTS programme.

Objectives:

To measure the total costs for patients enrolled in the programme and to estimate the economic and non-economic benefits of the programme to patients, family and the nation.

Methods:

All those chosen for the study and diagnosed with TB were started on treatment during the Jan – March 2006 formed the study population. So far, 260 patients have been interviewed. The study is in progress.

Socio-economic study of those suspected with TB present at the health care at Tambaram Sanatorium and in Kancheepuram district

Background:

Even though DOTS with the objective of providing decentralized diagnostic and treatment services is in place in Tamil Nadu for the last seven years, those with suspected TB from all over the state seek care at Tambaram Sanatorium. And over the years the out patient attendance has not come down.

For the patients, this involves traveling long distance, loss of income, stay during inpatient treatment and incurring expenditure. This may be due to lack of awareness or non acceptability of diagnostic and treatment facilities available locally among patients and or the referring practitioners.

Aim:

To understand the reasons and the socio economic profile of those suspected with TB coming to the Tambaram sanatorium during a three - month cohort period.

Methodology:

The study is being conducted at the government TB sanatorium Tambaram, Chennai. A semi-structured pre-coded interview schedule is used to collect relevant information regarding demographic, socio economic profile. In all, 2021 persons have been interviewed. The analysis is in progress.

Multi-Drug Resistance-TB (MDR-TB) management in the community – a field report from south India

Background:

Emergence of MDR-TB is a potential threat to the success of TB control. Patients diagnosed with MDR-TB in the study area, were managed by TRC under programme conditions.

Aim:

To document the experience in the management of MDR-TB patients identified in the RNTCP implemented rural area of Tamil Nadu.

Methods:

Diagnosis of MDR-TB:

For all TB patients registered for treatment under the RNTCP in the rural area, 2 additional sputum samples were collected within a week of starting the treatment. Patients were referred by an NGO if they suspected MDR-TB and 2 sputum specimens were collected at TRC for these patients. All specimens were processed at TRC for culture and drug susceptibility testing.

Treatment procedures:

Any patient identified with MDR-TB, was referred to TRC, Chennai, for initiating a second line treatment. Before registering for treatment, all patients underwent a detailed clinical, sociological assessment and laboratory investigations. They were started on an appropriate second line drug treatment regimen, based on drug susceptibility. After initiation of treatment, patients were advised hospitalization for at least a minimum period of one month, to monitor the drug tolerance pattern.

Results:

Patients identified with organisms resistant to both, INH and RMP (MDR-TB), with or without resistance to other drugs, were treated with one of the following regimen (Table 7a):

Table 7a: Drug Regimens

Regimen	Number of patients
S_3/K_3Ofl_7, Eth_7, Emb_7, Z_7	46
Individually tailored	20
Total	66

Among 66 patients, 12 were resistant to two drugs i.e. INH & RMP. In 34 patients, organisms were resistant to one or two of the first line drugs in addition to INH & RMP (S/EMB). In the remaining 20 patients, resistance pattern included second line drugs (Eth, OFX, and K).

APPLIED RESEARCH

Studies completed:

Pharmacokinetics of generic fixed-dose combinations of nevirapine, lamivudine and stavudine in HIV-1 infected adults in India

Background:

Generic Fixed Dose Combinations (FDC) of regimens containing two Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and one Non-NRTI (NNRTI) are widely regarded as crucial in the scaling up of AIDS treatment in developing countries.

Two triple drug combinations of nevirapine (NVP) and lamivudine (3TC) with either stavudine (d4T) or zidovudine (AZT) as the third drug are available as FDCs in the developing world. Since very limited data is available on the blood levels or pharmacokinetics of these drugs in Indian patients, a study was undertaken with the following aims:

Aims:

1. To obtain information on the pharmacokinetic profiles of NVP, 3TC and d4T in HIV-infected patients on treatment with FDCs in India.
2. To study the influence of immunological status, sex and body mass index (BMI) on the pharmacokinetics of these drugs.

Methods:

HIV – infected adults attending the outpatient clinic of the centre during October 2004 to September 2005 and undergoing treatment with generic FDC of antiretroviral drugs (NVP 200 mg /3TC 150mg/ d4T 30/40 mg or AZT 300 mg bi-daily) for a minimum period of two weeks, participated in the study. The study was conducted at the Government Hospital of Thoracic Medicine, Tambaram, Chennai. On the day of the study, serial blood samples were collected at different time points after administration of the FDC pill. Plasma NVP, 3TC and d4T were estimated by validated HPLC methods. Based on the plasma concentrations, certain pharmacokinetic variables were calculated using WinNonlin software.

Results:

Twenty nine HIV-infected patients took part in the study. Their baseline characteristics are given in Table 8. The steady state pharmacokinetics of NVP, 3TC and d4T are shown in Table 9. Peak and trough concentrations and exposure of NVP were higher in Indians than American and European populations. But it was similar to that reported in Malawians. The pharmacokinetic profile of 3TC and d4T in Indians was almost similar to that reported by others. The degree of immune suppression, sex and BMI did not have any impact on the pharmacokinetics of NVP, 3TC and d4T. Although, a significant difference in peak concentration of d4T between patients with CD4 cell counts = 200 cells/mm^3 (0.33µg/ml) and > 200 cells/mm^3 (0.53µg/ml), was observed ($P < 0.05$), these values are within the therapeutic range of the drug.

Table 8: Baseline characteristics of study participants

Characteristics	Value
Sex (No)	
Males	19
Females	10
Age (Years)	
Mean	36
Range	26-50
Body Weight (kg)	
Mean	52
Range	35-91
Height (cm)	
Mean	159
Range	140-173
BMI	
Mean	20.3
Range	14.5-33.0
Duration of ARTI (months)	
Mean	4.4
Range	1-17
CD4 counts (cells/mm^3)	
Mean	218
Range	25-684
$\geq$ 200 cells/mm^3 (No.)	16
<200 cells/mm^3 (No.)	13

Conclusion:

Adequate plasma concentrations of NVP, 3TC and d4T that are not influenced by the stage of immune suppression, gender and BMI observed in this study, are quite encouraging. Hence, if patients take regular treatment, chances of failure due to inadequate drug levels are low. The relatively higher steady state plasma NVP concentrations observed in Indian patients indicate the need to explore pharmacogenetic differences that could impact drug levels in different populations.

Table 9: Steady state pharmacokinetics of nevirapine, lamivudine and stavudine

	Mean ± SD				
Drug	C_{max} (µg/ml)	C_{min} (µg/ml)	T_{max} (hours)	AUC_{0-12} (µg/ml-hours)	t ½ (hours)
Nevirapine n = 26	8.50 ± 2.44	5.05 ± 2.04	1.38 ± 0.83	80.69 ± 26.85	29.82 ± 11.66
Lamivudine n = 27	2.39 ± 0.61	0.27 ± 0.13	1.5 ± 0.90	11.63 ± 2.99	4.48 ± 1.84
Stavudine n = 14	0.42 ± 0.18	0.025 ± 0.003	0.89 ± 0.40	1.45 ± 0.42	3.23 ± 1.92

C_{max}- peak concentration; C_{min}- trough concentration; T_{max}- Time to attain C_{max}; AUC-Area under the plasma concentration vs. time curve.

Studies in progress:

Screening for CYP2B6 (G516T) polymorphisms in HIV-infected patients in India

Background:

The use of combinations of antiretroviral drugs to provide potent ART, has dramatically improved the morbidity and mortality due to HIV infection and AIDS. Investigation of host genetic factors that impact both the efficacy and toxicity of ART, may aid in selecting the best regimen for individual patients. The non-nucleoside reverse transcriptase inhibitors, efavirenz (EFV) and NVP are used as a first-line treatment of HIV-infected patients in India along with nucleoside reverse transcriptase inhibitors.

Plasma concentrations of EFV are known for a high degree of inter-patient variability. Similar variability is observed with NVP. These drugs are metabolized by the cytochrome P450 2B6 (CYP2B6), an isoenzyme characterized by wide inter-individual variability in expression and activity in human livers *in vitro*. A CYP2B6 allelic variant in exon 4 (G516T, Gln172His), has been reported to influence EFV and NVP pharmacokinetics in Japanese, European-Americans and African-Americans. Identification of population differences in the frequency of polymorphism in the gene that encodes CYP2B6 is a matter of concern, since genetic differences among populations could lead to differences in antiretroviral drug concentrations, tolerability and outcome.

Information on the frequency of CYP2B6 polymorphisms in an Indian population and its association with EFV and NVP blood levels is lacking. It has therefore been planned to carry out pharmacogenetic studies with the following aims:

Aims:

1. To study the frequency distribution of CYP2B6 polymorphisms by genotyping for substitution at position 516 in exon 4 (G>T) in HIV-infected patients in south India.
2. To assess the influence of CYP2B6 polymorphisms on plasma concentrations of EFV and NVP.

Methods:

HIV-infected subjects receiving EFV (600mg once daily) or NVP (200mg twice daily) along with two nucleoside analogues for a minimum period of 15 days at the Government Hospital of Thoracic Medicine, Tambaram will be included. Plasma samples will be collected at 12 hours for EFV and 2 hours for NVP after drug administration. The drug concentrations will be analysed by High Performance Liquid Chromatography (HPLC). Genetic characterization of the CYP2B6 gene at position 516 will be performed by polymerase chain reaction – restriction fragment length polymrophism (PCR-RFLP) analysis using genomic Deoxyribonucleic Acid (DNA) extracted from whole blood. The primers, forward (5′ CTTGACCTGCTGCTTCTTCC 3′) and reverse (5′ TCCCTCTCCGTCTCCCTG 3′) will be used to amplify a 204 base pair product. The PCR product will be digested with

BsrI at 65°C overnight. Based on the number and size of the fragments, the subjects will be classified as GG, GT or TT genotype. Genotypes will be identified by the pattern of bands observed as follows:

GG	2 fragments, 152, 52 bp
GT	3 fragments, 204, 152, 52 bp
TT	1 fragment, 204 bp

The different genotypes will be correlated with 12-hour concentration in the case of EFV and 2-hour concentration in the case of NVP.

Fifty patients each receiving NVP and EFV will be included in to the study.

So far, genomic DNA has been extracted from 25 patients on NVP regimen and 15 patients on EFV regimen. PCR-RFLP analyses of these samples are in progress.

Pharmacokinetics of efavirenz during anti-TB treatment with rifampicin-containing regimens

Background:

EFV, a non-nucleoside reverse transcriptase inhibitor, has been recommended as a first line option in the ART and the preferential choice in TB and HIV co-infected patients. The available pharmacokinetic data provide evidence for only a 13-25 per cent reduction in EFV levels, when co-administered with RMP. This is lower than nevirapine (40 per cent) and protease inhibitors (80-95 per cent).

Despite few studies demonstrating favorable clinical outcomes in HIV-TB patients receiving EFV and RMP concomitantly, serious concerns have been raised regarding the adequacy of the conventional dose of EFV (600mg once daily) when co-administered with RMP—particularly in patients with higher body weight. At present there is no information available on EFV pharmacokinetics and the effect of RMP on EFV metabolism in Indian subjects. Therefore a study was planned with the following aims:

Aims:

1. To compare peak, trough levels and exposure of EFV in HIV and TB co-infected patients during and after completion of anti-TB treatment with RMP-containing regimens.
2. To correlate blood levels of EFV with body weight of patients.

Methods:

The study is being conducted in collaboration with the Government Hospital of Thoracic Medicine, Tambaram. The study participants will comprise of adult HIV-TB patients, undergoing treatment regularly with EFV and RMP containing antiretroviral and anti-TB regimens respectively, for a minimum of 1 week and willing to give informed consent. It is proposed to conduct the study amongst 30 HIV-TB patients.

All patients will be investigated on 2 occasions – once while receiving EFV and RMP-containing antiretroviral and anti-TB regimens, and next a month after completion of anti-TB treatment. During both the occasions, serial blood samples

will be collected at different time points after oral administration of EFV (600 mg). Estimation of plasma EFV will be carried out by HPLC. Based on the plasma concentrations of EFV and RMP at different time points, certain pharmacokinetic variables will be calculated.

The pharmacokinetic variables of EFV obtained during the anti-TB treatment (occasion I), will be compared with those obtained after completion of anti-TB treatment (occasion II). The per cent change in EFV pharmacokinetics in the presence of RMP, will be calculated. Correlation between pharmacokinetic variables of EFV and body weight will be calculated. So far, fifteen patients have completed the first occasion of the study.

Studies completed:

Candidal antibody response in serum and saliva of HIV-infected patients with and without oral Candidiasis

Background:

HIV-infected individuals are predisposed to recurrent oral candidiasis. A study was carried out to investigate the role of humoral immunity by comparing the concentrations of IgA and IgG antibodies to *Candida albicans* in whole saliva and serum samples from HIV-infected patients, with and without oral thrush.

Methods:

The study target comprised of 14 HIV seropositive patients with oral thrush (HIV+ oral thrush), 16 HIV seropositive patients without thrush (HIV+) and 13 healthy controls (HIV-).The anti candidal IgG titer was measured in saliva and serum using commercial ELISA kits.

Results:

In all the three groups, the IgG levels were higher in serum while IgA levels were higher in saliva. The HIV+ oral thrush group had significantly higher levels of candida specific IgA (45.3$\pm$ 4.1 Vs 31.2 $\pm$ 2.4) and IgG (31.5 $\pm$ 5.7 Vs 14 $\pm$ 1.4) in the saliva compared to normals ($p < 0.05$). There was no correlation between CD4 counts and IgG or IgA levels.

Conclusion:

These results suggest that HIV seropositive individuals are able to mount a good local antibody response. A defect in the mucosal humoral immune response in the oral cavity in HIV seropositive individuals does not appear to be responsible for the increased prevalence of oral candidiasis. High levels of candida specific IgA and IgG in saliva do not protect the patients from developing oral thrush. Further studies on cellular immunity have to be done to relate defective local immune response and the increased prevalence of oral candidiasis in HIV seropositive individuals.

Seroprevalance of Herpes simplex Virus –1 & 2 antibodies in HIV Positive and HIV negative individuals in south India

Background:

The Herpes Simplex Virus (HSV) is quite common through out the world. It is generally associated with oral (HSV-1) or genital ulcers (HSV-2) and is transmitted heterosexually. A large proportion of individuals with serologic infection with HSV

are asymptomatic. HSV-2 is a significant public health hazard because of its potential role as a cofactor in HIV transmission. A study was carried out to compare the sero prevalence of HSV-1 and HSV-2 infection in HIV sero positive and sero negative individuals in south India.

Materials and Methods:

A cohort of 70 HIV-infected and uninfected individuals, were included in this prospective study (35 HIV-infected and 35 HIV uninfected). All serum samples were randomized and assessed for HSV-1 and HSV-2 IgG and IgM antibodies with HSV type specific Enzyme Linked Immunosorbant Assay (ELISA).

Results:

In the HIV positive group, out of 35 individuals screened for HSV-1 antibodies, 34.3 per cent were positive for IgM and 80 per cent were positive for IgG antibodies. When screened for HSV-2 antibodies, the frequencies for IgM and IgG were 28.6 per cent and 60 per cent respectively. In the HIV negative group, out of 35 individuals screened for HSV-1 antibodies, the occurrence of IgM and IgG antibodies were 34.3 per cent and 71.4 per cent respectively, while the antibodies for HSV-2, showed prevalence rate of 17.1 per cent for both IgM and IgG. Using the rate chi-squared test, it was observed that there is a relationship between the presence of HSV-2 antibody and the HIV status. There is a significant difference between HIV positive and HIV negative individuals in prevalence of HSV-2 IgG antibodies ($P<0.05$).

Conclusion:

A higher rate of HSV-2 IgG antibody was found in HIV-infected individuals as compared to HIV negative individuals. This could have increased the risk for HIV acquisition.

Patients with TB have cross reacting antibodies against HIV-1

Some individuals produce antibodies that react with HIV-1 proteins, but are not diagnostic for HIV infection. Western blots from patients with TB, were analyzed for the presence of cross-reacting antibodies to HIV antigens. A total of 153 TB serum samples were analyzed for the presence of reactive bands against HIV antigens. All 153 patients who were HIV negative, showed varied bands to the HIV-1 antigens on western blot and Line Immuno Assay (LIA). Band appearance to the pol gene products (p-66, p-55, p-51, p-31) of HIV-1 appeared most frequently. A few bands were produced against the HIV-1 env proteins (gp-120 and gp-41). Bands against the gag proteins were also seen (p-24 and p-17). Cross-reactivity was seen more commonly with LIA than with western blot. A cladistic protein parsimony approach and a distance based neighbor-joining approach, were used to evaluate and identify the cross reacting antigens in *M. tuberculosis*. A phylogenetic tree was constructed between HIV-1 subtype C antigen sequences and *M. tuberculosis* CDC1551 antigen sequences.

It appears that TB patients could exhibit false positive antibody reactions against HIV antigens and that some *M. tuberculosis* antigens may have a similar parental gene as HIV. Patients with active TB have HIV-1 cross-reacting antibodies on western blot. This may interfere with the HIV-1 diagnostic testing. Further research is required to analyze the relation between HIV and *M. tuberculosis* antigens *in vitro.* Results of these studies may provide an insight on why such non diagnostic bands are frequent in HIV testing.

BASIC RESEARCH

Studies completed:

Evaluation of the diagnostic potential of RD-1 encoded CFP-10 antigen in TB

Background:

The 38kDa (Rv0934) has been extensively used in serodiagnosis, because it is species-specific. The use of 38kDa in serodiagnosis has yielded a sensitivity of 60 per cent in our laboratory and also in other studies. There is a consensus in the field of immunodiagnostics that multiple antigens must be used to enhance the sensitivity and that more and more species specific antigens are to be added to the 38kDa.

Aim:

To study the ability of species-specific antigen Culture Filtrate Protein -10 (CFP-10) to enhance sensitivity, especially when combined with the species-specific antigen 38 kDa.

Methods:

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

Disease groups:

1. Smear and culture positive patients with pulmonary TB (S+C+) (n = 262)
2. Smear negative and culture positive patients (S-C+) with pulmonary TB (n = 60)
3. Smear and culture negative, but radiologically diagnosed cases (S-C-) (n = 186)

Control groups:

1. Normal Healthy Subjects (NHS) (n = 160)
2. Other lung diseases (n = 76)
3. Disease control (n = 20)

Results:

Antibody levels of class IgG, IgA and combination, for the two polar groups, S+C+ and NHS for 38kDa and CFP-10 are shown in Table 10. With 38kDa antigen, out of 262 S+C+ TB sera, 158 were positive for IgG, giving a positivity of 60.3 per cent (S+C+). In the case of CFP-10, of 262 sera (S+C+), 133 were positive for IgG yielding a positivity of 50.8 per cent.

Among 160 control sera (NHS), four were positive for 38kDa giving a specificity of 97.5 per cent and three were positive for CFP-10 giving a specificity of 98.2 per cent. Some sera showed positivity for IgA, even though negative for IgG. The addition of IgA to IgG enhanced the sensitivity. Therefore, subsequent results were

expressed as IgG+IgA positivity. The sensitivity (G+A) of 38kDa in S+C+, S-C+ and S-C- cases were 76 per cent, 57 per cent and 76 per cent respectively. The values for CFP-10 were 71 per cent, 67 per cent and 45 per cent (Table 11). The antibody response to each antigen was complementary to the other. The use of RD1-encoded specific antigen CFP-10 enhanced the sensitivity of 38kDa. The combined sensitivity was 98 per cent in S+C+ and 78 per cent in S-C+, the highest sensitivity obtained in our laboratory with combination of antigens. Therefore this antigen promises to be a good candidate antigen for serodiagnosis.

Table 10: Sensitivity / Specificity in polar groups

Isotypes	38kDa antigen				CFP-10 antigen			
	S+C+ (n=262)		NHS (n=160)		S+C+ (n=262)		NHS (n=160)	
	No. +ve	%	No. +ve	%	No. +ve	%	No. +ve	%
IgG	158	60.30%	4	97.50%	133	50.80%	3	98.10%
IgA	88	33.60%	4	97.50%	109	41.60%	3	98.10%
IgG+IgA	199	75.90%	8	95%	186	71%	6	96.20%

Table 11: Positivity by combination of antigens

Category	38 kDa		CFP-10		38 kDa + CFP-10	
	IgG + IgA		IgG + IgA		IgG + IgA	
	No. + ve	% sen.	No. +ve	% sen.	No. +ve	% sen.
S+C+ (N=262)	199	75.9	186	71	257	98.09
S-C+ (N=60)	34	56.7	40	66.7	47	78.3
S-C- (N=186)	142	76.3	83	44.6	146	78.4
Other lung diseases (N=76)	2	97.4	3	96.1	5.	93.43
Disease control (N-20)	1	95	1	95	2	90
NHS (N=160)	8	95	6	96.2	14	91.2

% sen. Percentage sensitivity
S+C+ Smear positive; Culture positive group
S-C+ Smear negative; Culture positive group
S-C- Smear negative; Culture negative group
NHS Normal Healthy Subjects

Regulatory role of HLA-DR2 on macrophage phagocytosis and perforin positive cells in pulmonary TB

Background:

The study is part of the ongoing project on the role of HLA-DR2 on immune functions in pulmonary TB.

Aim:

To understand the role of HLA-DR2 gene on innate immune functions such as macrophage phagocytosis with live *M. tuberculosis* and perforin positive cells in pulmonary TB.

Methods:

The study subjects included 70 pulmonary TB patients and 70 normal healthy volunteers. DNA typing of HLA-DR, enumeration of *ex vivo* perforin positive cells by flowcytometry, macrophage phagocytosis with live *M. tuberculosis* was carried out on patients and normal subjects.

Results:

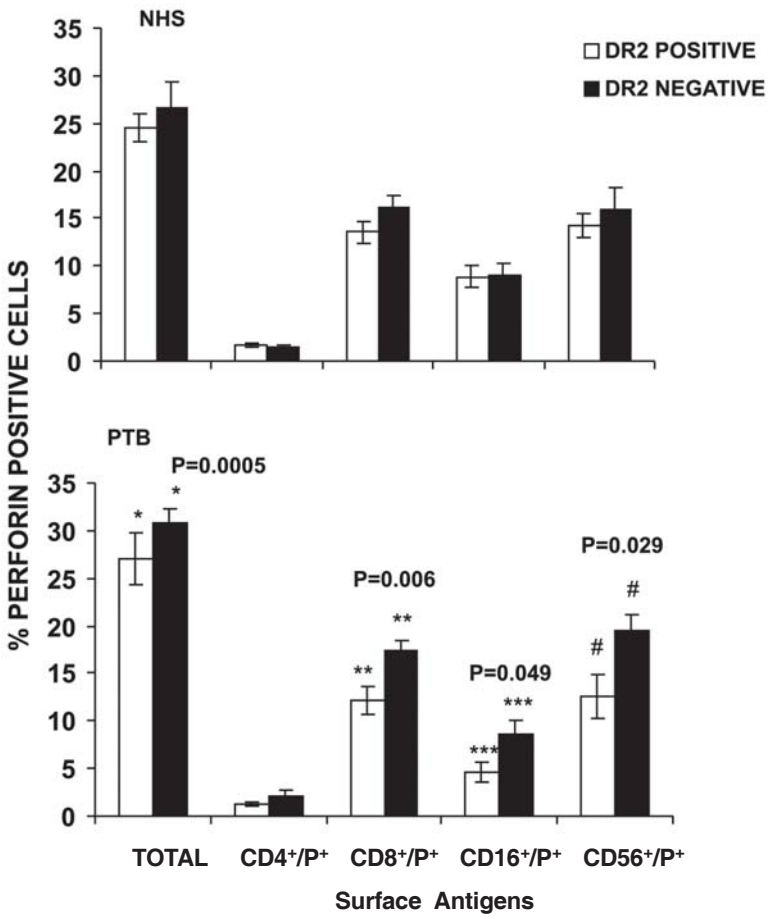
Macrophage phagocytosis:

A trend towards an increased percentage macrophage phagocytosis was observed in normal subjects with HLA-DR1, as compared to subjects with non–DR1 antigens ($p=0.07$). Significantly decreased macrophage phagocytosis was observed in normal persons with –DR3 ($p=0.04$) and –DR10 ($p=0.0001$) antigen than –DR3 and –DR10 negative individuals. Whereas, no such difference in the macrophage phagocytosis was observed in pulmonary TB patients. Moreover, analysis on the phagocytosis of –DR1, -DR3 and –DR10 positive normal subjects revealed a trend towards an increase in phagocytosis with DR1/DR2 combination and a decrease with DR2/DR3 (DR 1/2 vs. DR 2/3) ($p=0.03$) and DR2/DR10 combinations.

Ex vivo perforin positive cells:

Significantly decreased overall intracellular perforin positive cells were observed in HLA-DR2 positive TB patients, than non-DR2 group ($p<0.001$). Further analysis on intracellular perforin positive cells with various surface markers, revealed a significantly low perforin positive CD8 ($p<0.01$) CD16 ($p=0.049$) and CD56 ($p=0.029$) cells in HLA-DR2 positive pulmonary TB patients than non-DR2 patients (Fig.3). However, no such decrease was observed in normal subjects with and without -DR2 antigen.

Fig.3: Intracellular perforin positive cells and their immunophenotype in HLA-DR2 positive and -DR2 negative subjects in NHS and PTB



Conclusion:

The present study suggests that HLA-DR1, especially HLA-DR1/DR2 combination is associated with higher macrophage phagocytosis. HLA-DR3 and –DR10 are associated with lower phagocytosis. DR2/DR3 combination is associated with lower phagocytosis in normal subjects. Moreover, HLA-DR2 positive pulmonary TB patients showed decreased perforin positive cells. This suggests that HLA-DR2 influences the innate immunity to *M. tuberculosis* infection.

Human Leucocyte Antigen (HLA) and non-HLA gene polymorphism studies in HIV and HIV-TB patients (ICMR Task Force project, funded by ICMR, New Delhi).

Background:

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

Aim:

To find out whether HLA genes, HLA haplotypes and non-HLA genes are associated with the susceptibility or resistance to HIV and HIV-TB.

Methods:

The study subjects comprised of HIV negative TB negative (HIV-TB-) (n=150), HIV negative TB positive (HIV-TB+) (n=150), HIV positive TB negative (HIV+TB-) (n=150), and HIV positive TB positive (HIV+TB+) (n=150) groups. HLA-A, -B antigen were serologically determined. HLA-DR and –DQ and various non-HLA gene polymorphism were studied by PCR based DNA typing.

Results:

A trend towards a decreased antigen frequency of HLA-A11 is observed in HIV and HIV-TB patients. Moreover, an increased antigen frequency of HLA-B40, -DR2 was seen in HIV and HIV-TB patients than the control subjects. The above trends are based on 100 subjects in each group.

Performance of the Lysogenic phage Che12 LRP Construct phAETRC16 in sputum samples

Background:

Luciferase Reporter Phage (LRP) construct (phAETRC16) from Che12 was developed for increasing the sensitivity of the rapid diagnostic LRP assay.

Aim:

Evaluation of phAETRC16 for the diagnosis of TB

a. In comparison with phAE129 using spiked sputum samples.
b. On 0 day and 7th day using conventionally decontaminated sputum deposits treated with phagebiotics.

Method:

Smear negative sputum samples were spiked with *M. tuberculosis* suspension.

Readings were taken the same day, viz. six hours post infection with LRPs.

Sputum deposits obtained after processing by Petroff's method were suspended in 7H9 medium containing phagebiotics. Phagebiotics is the cocktail of three phages capable of controlling normal flora that survive the action of four per cent NaOH after Petroff's decontamination. About 122 smear positive sputum samples were tested – out of which 10 sputum samples gave RLU more than 500. These were considered as positive for LRP and analyzed.

Results:

Out of 30 spiked sputum samples tested, 12 gave RLU more than the cut off value of 500. The $\log_{10}$ values are plotted in the graph (Fig.4) of the 12 samples, 9 showed higher readings with phAE129, and the remaining 3 showed no significant difference.

Statistical analysis using Wilcoxon Signed Ranks Test at 95 per cent significance level showed that there was a statistically significant difference between the spiked sputum readings of phAE129 and phAETRC16 readings ($p < 0.01$). The light out put of phAETRC16 on zero day and seventh day was compared and the readings of the 10 sputum samples were plotted as $\log_{10}$ in the graph (Fig.5). Comparison of results showed only a marginal increase on the seventh day. The statistical analysis showed that there was no significant difference.

Conclusion:

a) phAE129 was found to perform better than phAETRC16 in spiked sputum samples.

b) A marginal increase in light output was observed on the seventh day in comparison with the zero day as expected with the lysogenic phage construct.

Fig.4: Log_{10} values of RLUs of phAETRC16 vs phAE129 in spiked sputum samples

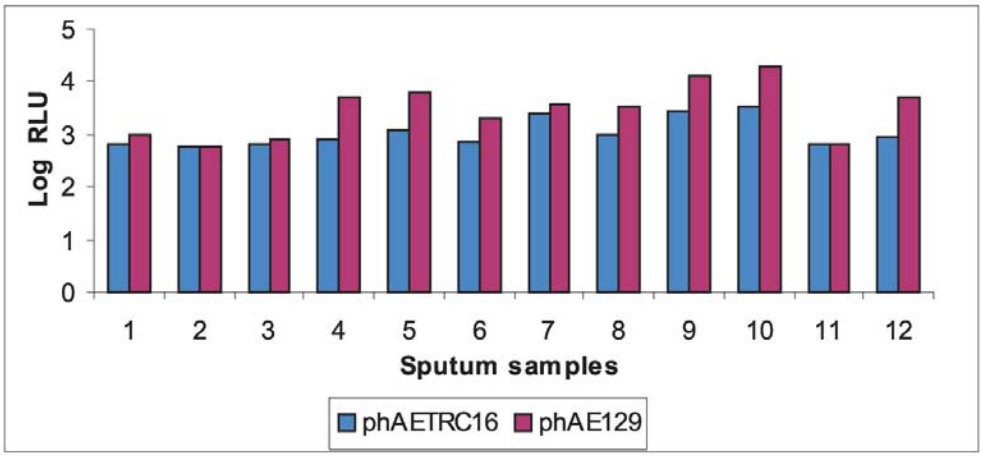
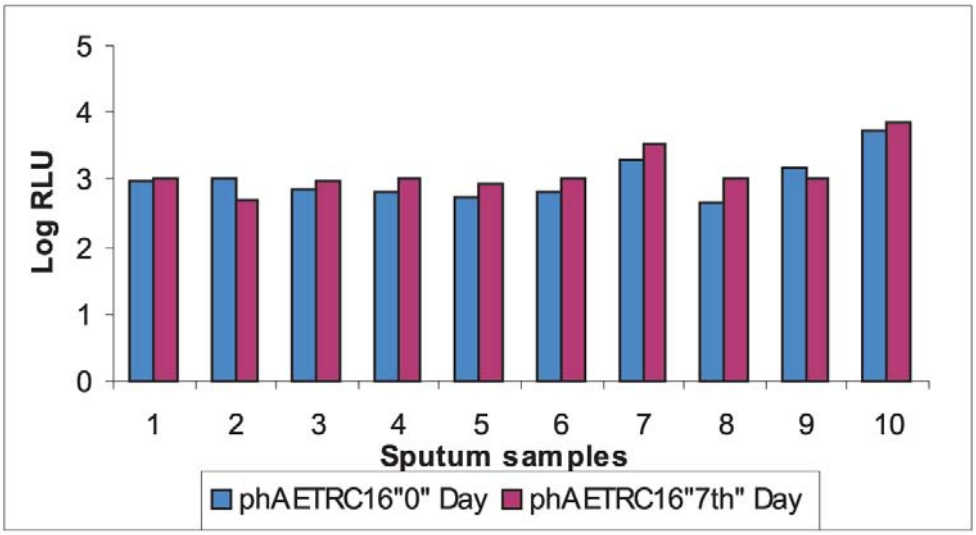


Fig.5: Log_{10} values of RLUs of phAETRC16 on 0 day and 7th day in Sputum samples



Studies in progress:

Over expression of MSMEG3682', a protein kinase from *M. smegmatis* associated with its morphological changes

Background:

The genome of *M. smegmatis* (sequencing at TIGR), has 18 putative serine threonine kinases. While the genome of *M. tuberculosis,* contains 11 putative serine threonine kinases. MSMEG3682' of *M. smegmatis,* was the only protein homologous to both PknF and PknI of *M. tuberculosis.* Both of which are involved in cell division

in *M. tuberculosis*. We have reported for the first time a functional kinase from *M. smegmatis* and revealed its role in cell division.

Aims and Objectives:

To express the MSMEG3682 protein in *M. smegmatis* mc^2155 under the control of acetamidase promoter, and study the effect of induction on growth, viability and cell morphology.

Methodology:

Standard molecular biology techniques were followed for the DNA manipulations and merodiploid strains constructed. For the induction experiments, 0.2 per cent acetamide (final concentration), was added to LB broth or solid media containing 0.05 per cent Tween 80. Growth was assessed by measuring OD_{600} and viability by measuring CFU's. Cells were visualized by scanning electron microscopy. A total of 100 cells of each of the strains were measured for their length using the ANALYSIS software. These were then classified based on their sizes (in µm) into groups.

Results:

MSMEG3682 was over expressed in *M. smegmatis* mc^2155, under the control of an inducible acetamidase promoter. Its effect on growth and viability was also studied. Changes in cell morphology were analyzed by scanning electron microscopy. Growth retardation was observed with the induced and uninduced merodiploid strain, compared to the wild type strain by three and 1.1 fold reduction (Fig.6). A corresponding difference in viability of three log and 1.7 log was found when plated on acetamide containing - LB plates (Fig.7). There was extensive clumping and cell lysis when merodiploid strain was grown beyond 30 hours. Scanning electron microscopy revealed irregular cell structure marked with bulb-like protrusions along the length of the cell or at the end of the induced merodiploid strain (Fig.8). Control strains failed to show differences in growth, viability and cell morphology.

Fig.6: Growth kinetics of merodiploid strain over expressing MSMEG 3682'

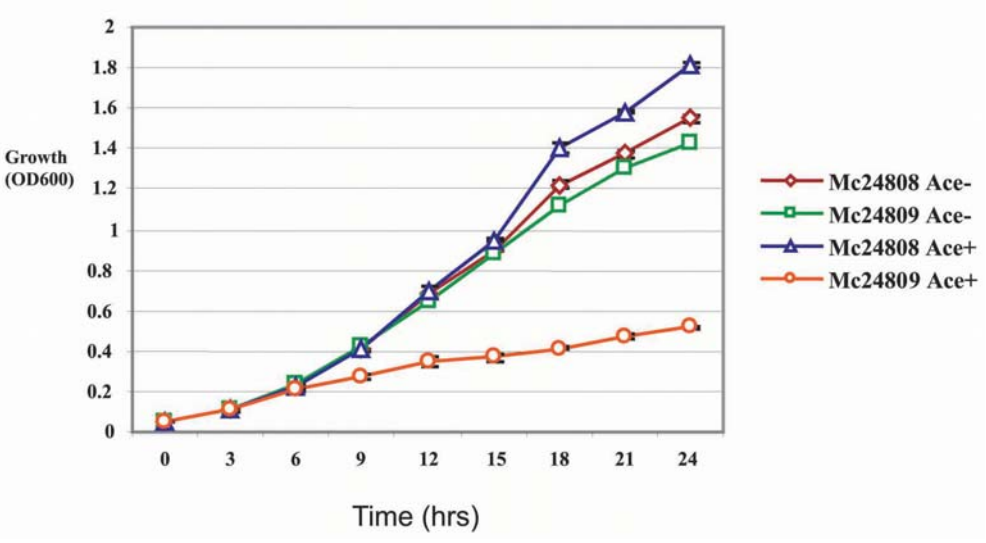
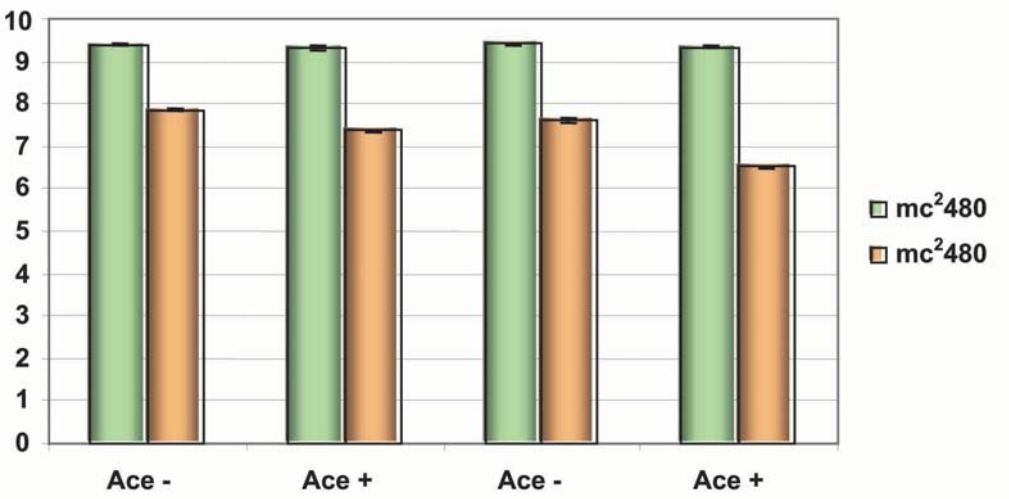
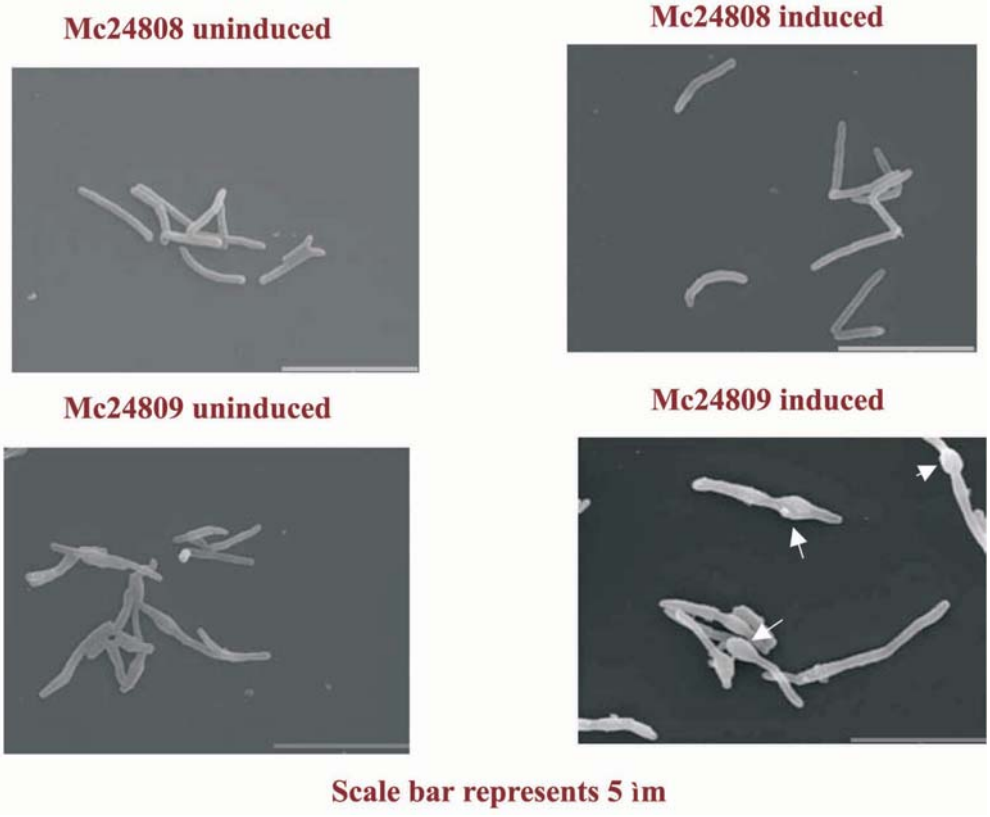


Fig.7: Viability of merodiploid strain over expressing MSMEG 3682'



Growth conditions in liquid and solid media

Fig. 8: Over expression of MSMEG3682' and gross morphological changes by SEM



Conclusion:

The study findings confirm our hypothesis that MSMEG3682' participates in controlling cell division and cell structure.

Protein Kinase E of *M.tuberculosis*: Implications in Nitric oxide Stress and Redox Regulation

Aims:

1. To create knock out mutants of pknE, pknF and pknL in *M.bovis* BCG by specialized transduction.
2. To study and see whether these genes are essential for the intra cellular survival of *M. bovis* BCG in THP1 macrophage cell line.
3. To study the role of pknE in stress response.

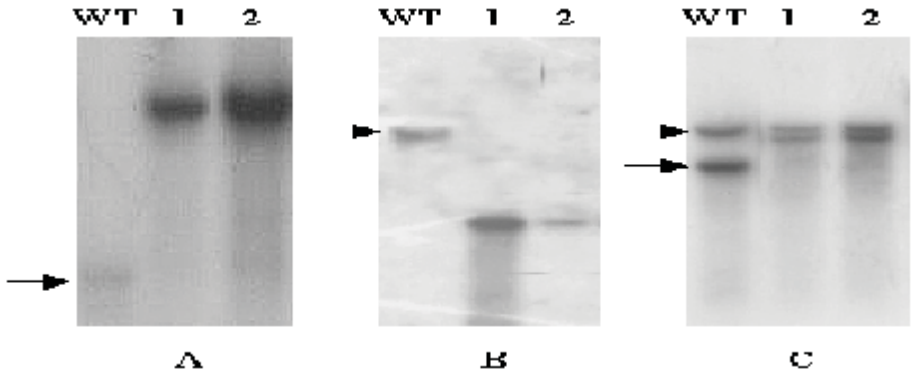
Methods:

Construction of gene disrupted strains of STPKs in *M.bovis* BCG and *M. tuberculosis*:

The pknE gene disrupted strains were generated by specialized transduction in *M. tuberculosis* and *M.bovis*. Briefly, the N-terminal and C-terminal flanking regions corresponding to 813 bp and 719 bp of pknE, were PCR amplified from the genome of *M. tuberculosis* H37Rv. These PCR products were cloned into pCR 2.1 (Invitrogen, USA), sequenced and subcloned into pJSC347. The SpeI and XhoI site in the first multiple cloning sites and the XbaI and StuI in the second multiple cloning sites, were used to insert the N-terminal and C-terminal flanking regions respectively. The Allelic Exchange Substrate (AES) was then cloned into the shuttle phasmid, phAE159 by *in vitro* packaging in *E. coli* resulting in ph159-PKNE. The recombinant phage DNA was electroporated into *M. smegmatis,* and high titres of the knock out phage was used to transduce *M. bovis* BCG-Pasteur in different bacilli-to-phage ratios. A genomic DNA was extracted and southern blot analysis of MluI digested DNA of both the strains was performed, using the N-terminal flanking region as the probe (result for the pknE mutant in *M. bovis* BCG is shown in Fig.9a).

A)pknE mutant was also constructed in *M. tuberculosis*, H37Rv. Similarly pknF and pknL disrupted strains were constructed in *M. bovis*. (Fig.9 b & b).

Figs. 9 a, b & c: Disruption of *pknE*, *pknF* and *pknL* in *M.bovis* BCG-Pasteur. Bands in the wild type lanes are indicated by black arrows (→). Southern Blot analysis of (A) the)*pknE* mutants (1 and 2) WT-4.1 kb, mutant-12.54 kb (B) the)*pknF* mutants (1 and 2) WT-7.524 kb, mutant-1.481 kb (C) the)*pknL* Mutants (1 and 2) WT-4.893 kb/9 kb, mutant-7.429 kb/9 kb.



Results:

PknE, *pknF* and *pknL* are non essential genes in *M.bovis* BCG-Pasteur and the corresponding mutants show different growth profiles *in vitro* and in a macrophage model of infection. *In vitro* growth kinetics showed that the *pknE* and *pknL* mutants were unaltered in their growth characteristics, when compared to wild type BCG. On the other hand, the *pknF* gene disrupted strain exhibited lower OD_{600} values, indicating that it was impaired in *in vitro* propagated cultures (Fig.10).

The human macrophage model of infection, namely THP-1, was used to assess the growth and multiplication of the mutant. Although no significant differences were seen with the *pknE* mutant when compared to the wild type strain, both the *pknF* and *pknL* gene disrupted strains showed reduced rates of growth and multiplication within macrophages (Fig.11).

Fig.10: Growth kinetics of the Δ*pknE*, Δ*pknE*: *egfp*, Δ*pknF*, Δ*pknL* (BCG mutants) compared to Wild type *M.bovis* BCG-Pasteur

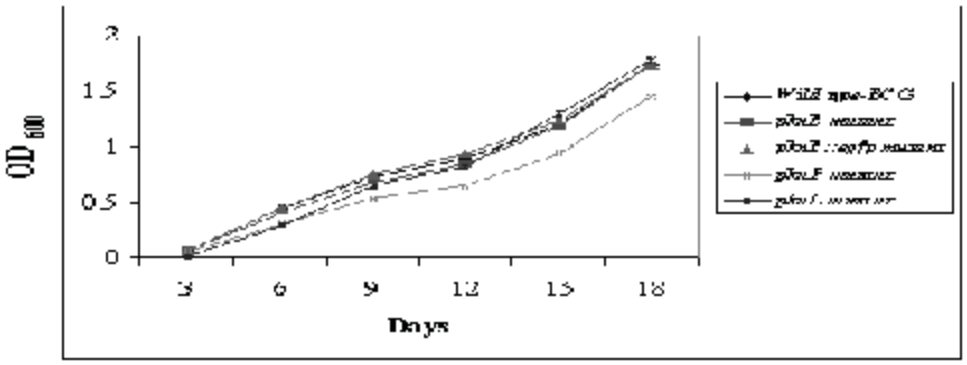
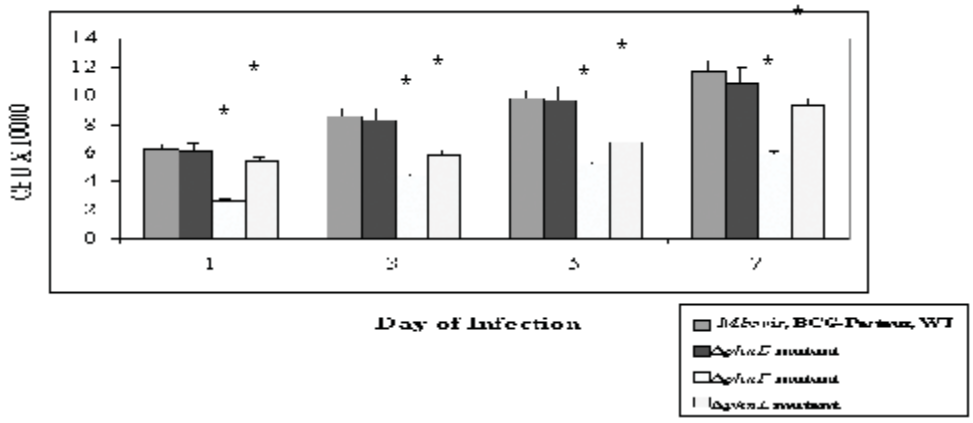


Fig.11: Macrophage survival assays for the Δ*pknE*, Δ*pknF and* Δ*pknL* mutants of *M.bovis* BCG-Pasteur in a THP-1 model of infection. Colony forming units estimated at specified time points.
***p<0.05.**



The Δ*pknE* mutant of *M. tuberculosis* was tested against a variety of chemical compounds to determine whether there were any differences when compared to the wild type or the complemented strains.

The Δ*pknE* mutant was sensitive to hydrogen peroxide when compared to the wild type strain. But this difference was not statistically significant. On the other hand, the mutant was resistant to varying degrees to nitric oxide donors added to *in vitro* grown cultures of the wild type, mutant and complemented strains.

Molecular Epidemiology of *M. tuberculosis*

Background:

The amplitude of the TB problem world-wide and the global traffic requires the application of effective approaches to decipher the portable genotype patterns of this region, in comparison with the global patterns of disease transmission. The genetic information resulting from high prevalence areas such as ours would prove useful for defining phylogenetic links that exist with TB genomes, and also for constructing models of genome evolution. This will also help elucidate the evolutionary history of ancient tubercle bacilli.

Spoligotyping which interrogates the Direct Repeat (DR) locus of *M. tuberculosis* has the potential to identify the global distribution of the major clades of the *M. tuberculosis* complex. Spoligotyping is a PCR-based method dependent on hybridisation patterns of *in vitro*-amplified DNA with multiple spacer nucleotides. This region contains multiple short 36-bp direct repeats (DRs) and non-repetitive spacers, which are 36 to 41 bp in length, interspersed between the DRs. Spoligotyping is a rapid method that allows large numbers of isolates to be handled in a short time. The DRs are extremely well conserved among *M. tuberculosis* complex strains, making spoligotyping a specific method of detection of *M. tuberculosis* complex members.

Aims:

1. To spoligotype 1200 *M. tuberculosis* isolates.
2. To compare the genotype of the *M. tuberculosis* isolates from south India with that of the genotype of *M. tuberculosis* isolates from other parts of the world using the global spoligo database.
3. To identify the lineage of *M. tuberculosis* isolates from the Tiruvallur area.

Materials and Methods:

A standardized international protocol was used for spoligotyping. This is in accordance with the protocol supplied by the Isogen Bioscience BV, Manarssen, and instructions of the manufacturer. All spoligotype patterns were coded using the octal code system. These were then referred to a standardized international database of spoligotype patterns, SpolDB3 available at www.pasteur-guadeloupe.fr/tb/spol3. This was to determine whether each pattern had been previously reported.

Results:

Hundred different genotype clusters were identified with at least two different patients each who had the same spoligotype pattern. A total of 1038 (85.4 per cent) TB patients had a clustered isolate and 177 (14.6), had isolate with a unique spoligotype pattern. There was a median of three patients per cluster (range 338 patients). The spoligo patterns of all the isolates were compared to Spol $DB_{3,}$

available at www.pasteur-guadeloupe.fr/tb/spol3. This was to determine whether each pattern had been previously reported.

We found 42 clusters with spoligotypes matching the Spol DB_3 (Fig.12). There were 23 unique isolates with spoligo patterns, which matched Spol DB_3 database. Fifty eight clusters had spoligotypes which did not match the Spol DB_3 database. These are considered as clusters of orphan isolates. Among these orphan clusters, 51 clusters were East African Indian (EAI) variants, 4 variants of Latin American Mediterranean or Harlem strains, and 2 clusters of 2 strains each, variants of the Beijing variants. There were 154 orphan strains, not involved in clusters. Among the orphan strains, 4 were not the descendents of any of the 4 lineages described by Baker *et al.* and they were totally orphan.

Fig.12: Clustered Spoligo types matching Spol DB 3.0

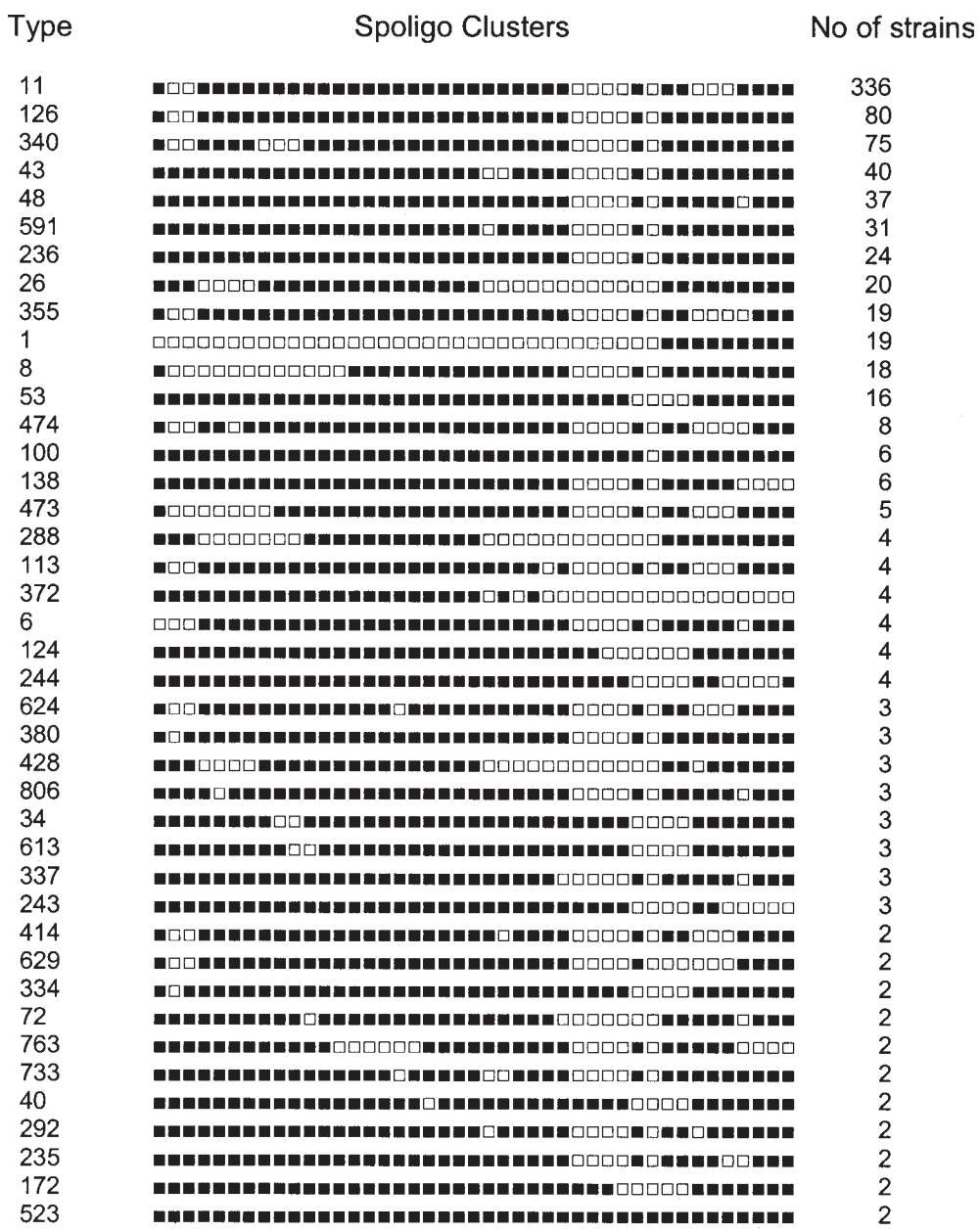


Table 12: Classification of spoligotype patterns into major phylogenetic lineages

Major phylogenetic lineage	Number of spoligotype patterns	Percentage of spoligotype patterns	Number of Isolates	Percentage of Isolates
Beijing (I)	1	(0.4)	20	(1.7)
Beijing variants (I)	9	(3.3)	12	(1.0)
Delhi prototype (III)	3	(1.1)	26	(2.1)
Delhi variants (III)	20	(7.2)	28	(2.3)
Manila prototype (IV EAI)	1	(0.4)	1	(0.1)
Manila variants (IV EAI)	194	(70.0)	1034	(85.1)
Δpks 15/1 (II)	41	(14.8)	83	(6.8)
Unknown	8	(2.9)	11	(0.9)
Total	277	(100.0)	1215	(100.0)

Groups (I, II, III, IV) Baker L. *et al*. 2004

The distribution of different spoligotype patterns into major phylogenetic groups is shown in Table 12. Most (70.0 per cent) of the spoligotype patterns were in Group IV, East African Indian, PGG1 (1), followed by the isolates in the Euro-American lineage (14.8 per cent) and the Delhi genogroup variants (7.2 per cent). Beijing lineage accounted for 20 prototype and 12 strains of Beijing variants, comprising about 2 per cent of the total isolates. Even more strikingly, 74.0 per cent of the clusters and 88.5 per cent of the clustered TB patients, had a spoligotype pattern in Group IV, followed by 14 per cent of the clusters and 5.4 per cent of the clustered TB patients in the Euro-American lineage.

Among the EAI group consisting of 1034 isolates, 30 per cent were of EAI class three, with the spoligo pattern of all spacers present, except 2-3, 29-32, 34, 37-39 spacers. Four per cent of the isolates belonged to EAI class 1 with all other spacers present, except spacer nos. 29-32, 34, and 40. The rest of the EAI isolates were variants of these 2 classes, yet to be defined.

Conclusion:

Our results showed that the major clade present in Tiruvallur belonged to the Group IV of Baker's classification that is named East-African Indian Lineage and close to the spoligo pattern of Manila isolates with the deletion at RD239.

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. This is due to many factors. Immuno-prophylactic measures using vaccines, is an alternative approach for control. A limited number of attempts to screen human responses to separated antigens have demonstrated that there are still numerous uncharacterized antigens

of various molecular masses to be evaluated. Moreover, a systematic approach to test the antigens purified by two-dimensional (2-D) preparative separations in human subjects, has not been attempted so far.

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) selected from families where there is at least one case of sputum positive pulmonary TB living in the same household. TB was ruled out in this group during the time of blood collection and hence considered "Protected".
2. Newly diagnosed adult pulmonary TB cases in the age group of 18-50 yrs. They form the "susceptible" group.

The methods:

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

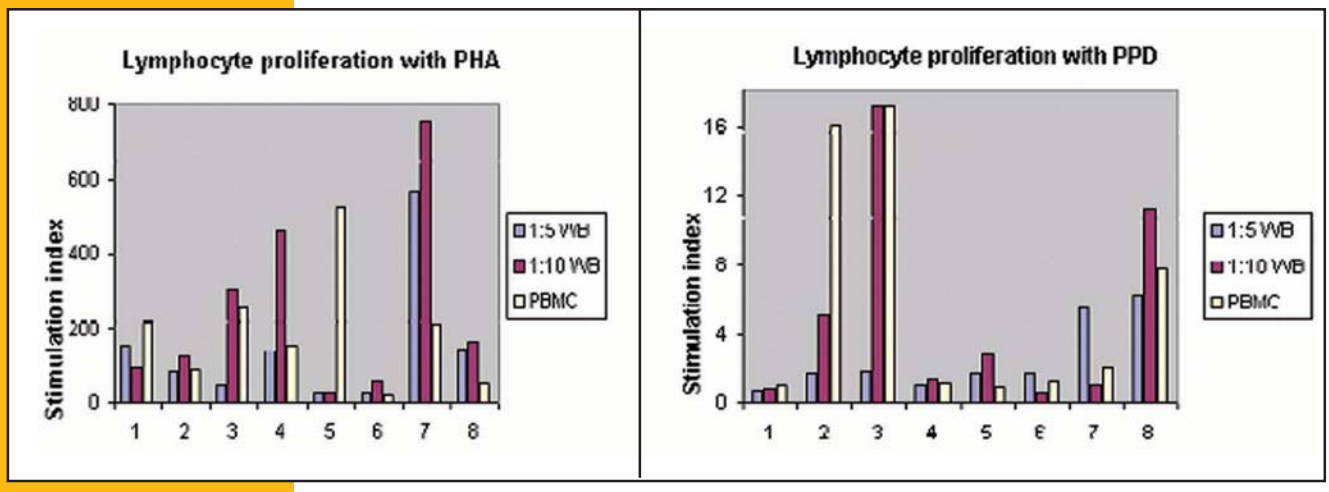
Results:

Using the preparatory two -dimensional approach, the *M. tuberculosis* secreted proteins have been separated into 600 fractions. Of these, fractions having at least 50μg or more were selected for further testing (347 fractions). Since a large number of fractions will have to be tested in each blood sample, we have standardized a whole blood (1:10 dilution) assay, for proliferation and IFN-γ secretion (Fig. 13 a & b). It has been planned to compare at least 10 HHC and 10 TB subjects. So far five HHC and six TB samples have been tested.

Fraction showing differential stimulation and IFN-γ production, in the "protected" (HHC) and "susceptible" (TB) subjects, will be selected for further characterization.

Fig 13: Lymphocyte proliferative response in whole blood

a **b**



Construction of luciferase reporter phages expressing FFlux gene driven by dormancy inducible conditional promoters

Background:

Early diagnosis of TB among the HIV-infected and general population is essential in reducing the morbidity and mortality. Over the past decade, LRPs have been developed. These show great promise in diagnostic microbiology. Conventional LRPs from lytic phages such as D29 and TM4 used in LRP assay, however highly specific, lack sensitivity. Hence it was hypothesized that temperate phage infecting *M. tuberculosis* if used in the construction of Reporter Phage System, would bring about a sustained light output. Moreover, most of the TB cases among the HIV-infected population result from the reactivation of latent bacilli and so LRP with a dormancy inducible promoter would identify viable, but not cultivable (VBNC) population of *M. tuberculosis.*

Aim:

To construct LRPs with dormancy inducible promoter driving Fflux gene.

Method:

Suitable cosmid vector with luciferase gene driven by hsp60 promoter was developed initially. Four efficient mycobacterial dormancy inducible promoters were identified and amplified from the H37Rv genome. A 368 bp PCR product of DevR promoter, 206 bp PCR product of Isocitrate-lyase promoter, 306bp PCR product of Nark2 promoter and a 220bp PCR product of Alpha Crystalline Protein (ACR) promoter, were cloned into the cosmid vector replacing hsp60 promoter individually. The presence of these conditional promoters was confirmed by sequencing the cosmid constructs. Using *Not*1 site for cloning and lambda packaging, a functional LRP (phAETRC101) was developed from phAE159, a temperature sensitive mutant of TM4. Using the same strategy, similar constructs with dormancy inducible promoters from the temperate phage Che12, were developed.

Results:

LRP was successfully constructed using Che12 with Isocitrate-lyase promoter expressing Fflux gene (phAETRC21) and it gave a four-digit RLU reading with *M. smegmatis* mc^2155 with integration time of 10 seconds. LRP construct with phAE159 having the alpha crystallin protein driving the luciferase gene was also developed (phAETRC102). The kinetics of phAETRC21 and phAETRC102 with *M. smegmatis* mc^2155 was studied. Kinetics of phAETRC102 was studied both at 30°C and at 37°C to define the ideal temperature for sustained light output (Table 13). The RLU of phAETRC102 was greater than the Che12 based LRP construct phAETRC21 with *icl* promoter producing maximum light at 30°C (Fig. 14). Kinetics of these constructs was studied and compared with phAE129 (D29 based LRP) in a clinical isolate of *M. tuberculosis*. The RLU of phAETRC102 was greater than the rest of the two LRPs compared (Table 14 and Fig. 15).

Conclusion:

LRP constructs with dormancy promoters driving the *Fflux* gene show promise for use in rapid diagnostic assay for TB.

Table 13: Kinetics of LRPs at 30ºC and 37ºC in *M. smegmatis*

	30 Mins	3 Hours	6 Hours	24 Hours
phAETRC21	5730	31129	69825	10334
phAETRC102 at 30 degree C	118825	560000	644033	83766
phAETRC102 at 37 degree C	81570	156980	39293	560

Fig. 14: Kinetics of different LRP constructs in *M. smegmatis* mc2155

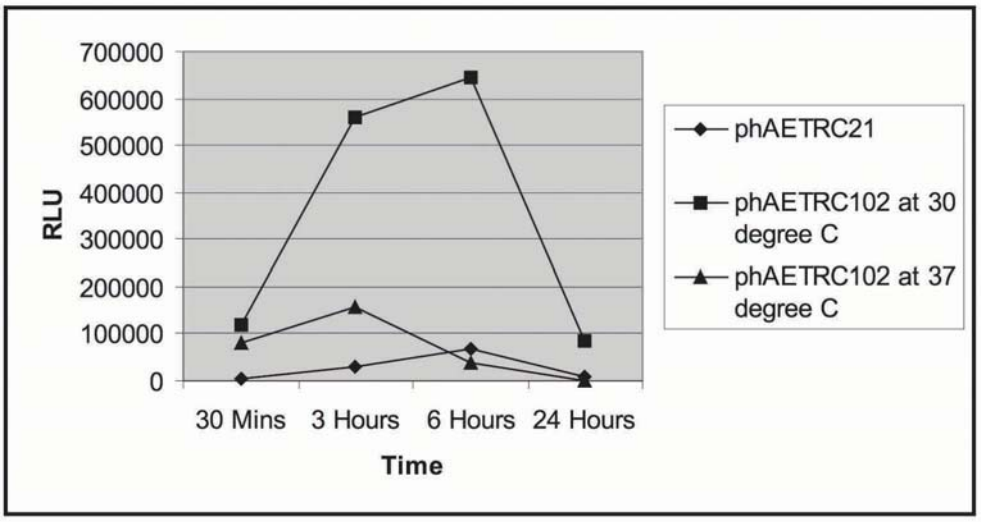
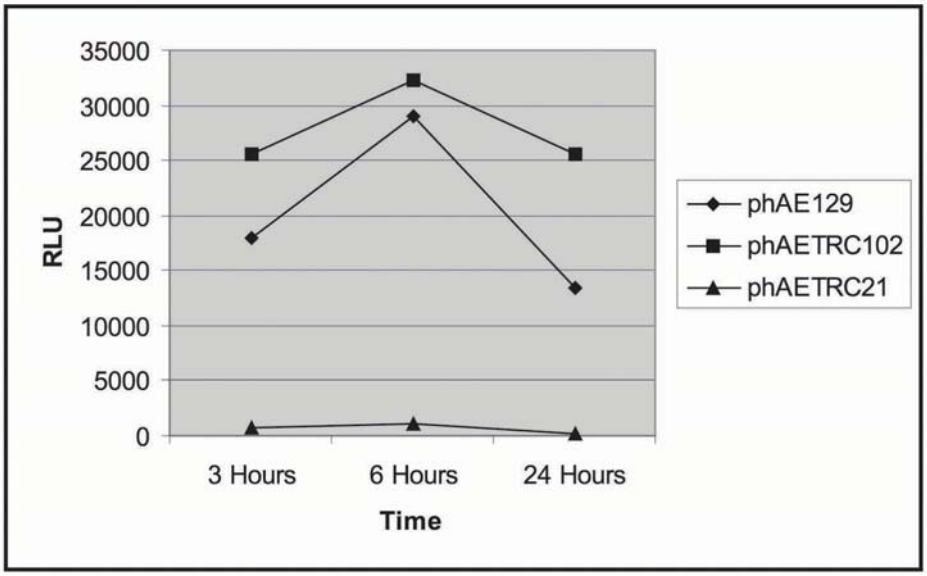


Table 14: Rapid diagnostic assay for tuberculosis

F9621	3 Hours	6 Hours	24 Hours
phAE129	17913	29046	13397
phAETRC102	25569	32271	25579
phAETRC21	698	1041	178

Fig.15: Kinetics of different LRP constructs in *M.* tuberculosis clinical Isolate No. RF 9721



An alternative method of sputum processing to detect *M. tuberculosis*

Background:

Conventional sputum processing by modified Petroff's method involves treating sputum specimens with 4 per cent NaOH, which is deleterious to tubercle bacilli to some extent. An alternate bio-friendly method that is mild on tubercle bacilli and at the same time harsh on normal flora exhibiting high mucolytic activity should result in increased detection of TB cases. In addition, it should help to improve the sensitivity of rapid diagnostic procedures as well. Chitin is present ubiquitously in the animal kingdom and is biofriendly. Its mucolytic activity and decontaminating ability could prove to be useful in developing an alternate sputum processing method.

Aim:

To develop a novel bio friendly sputum processing method, using chitin to detect tubercle bacilli.

Method:

A total of 120 samples were collected and divided into two parts. After randomization, one aliquot was processed with chitin-H_2SO_4 and another with modified Petroff's method as the gold standard. Both the deposits were inoculated onto LJ medium and randomized. Deposits were also inoculated onto blood agar medium to evaluate the effect of chitin on growth of normal flora.

Results:

Only 6 samples resulted in the growth of normal flora, surviving the action of chitin. Fifteen samples still showed the presence of normal flora when subcultured on blood agar (Fig.16). When smear results were compared with that of culture, Petroff's method performed better (kappa-0.77) as compared to Chitin method (kappa-0.67). Sensitivity of chitin processing method was 84 per cent and the specificity was 87 per cent. Culture results of chitin method showed an agreement of 85 per cent compared to the Petroff's method. The culture positivity of the smear negative samples was found to have statistical significance ($p<0.001$) (Fig.17).

Conclusion:

Chitin has better mucolytic activity and decontaminating ability than 4 per cent NaOH. The *M. tuberculosis* retrieval capacity of both methods is the same. Hence Chitin can be an ideal alternative to NaOH for sputum processing.

Fig. 16: Growth of normal flora on blood agar medium

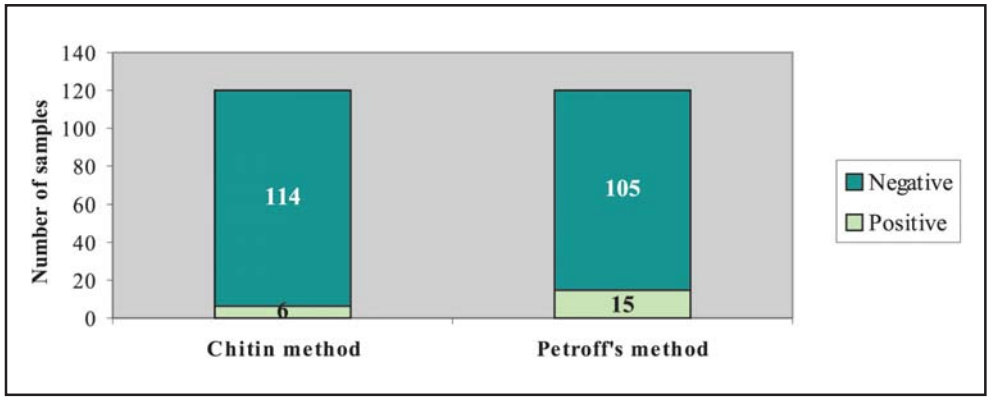
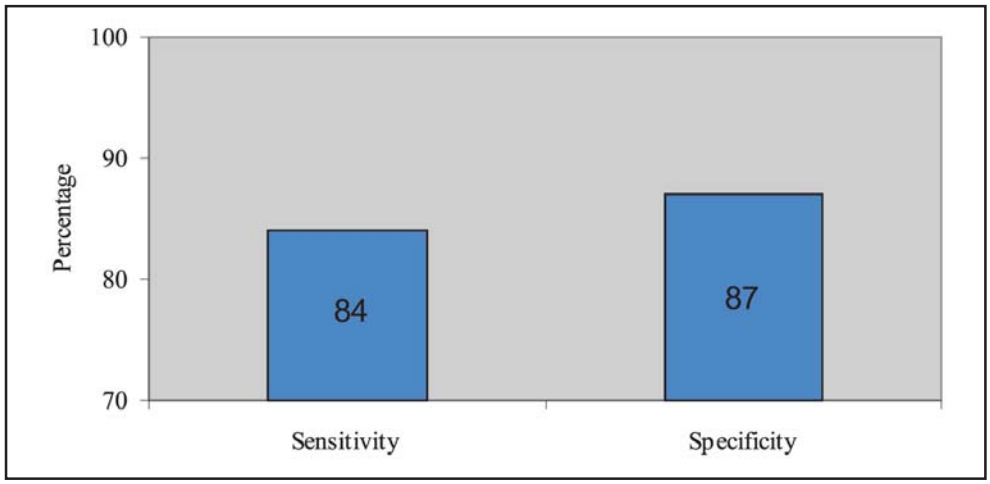


Fig.17: Sensitivity and specificity profile



Rapid screening of plant essential oils against *M. tuberculosis*

Background:

The resurgence of TB is one of the most serious public health challenges of the 21st century. The World Health Organization has given priority to the control of TB and prevention of the spread of drug resistant strains. There is an urgent need to discover novel anti-TB agents, especially from natural sources because of the increasing resistance of mycobacteria to the classic anti-TB drugs. The antimicrobial activity of plant oils and their extracts have been recognized for many years. Searching anti-TB compounds from plant essential oils, is a novel approach leading to the identification of new active molecules.

Luciferase Reporter Phage Assay is a rapid, inexpensive and less laborious method for high through screening of compounds against *M. tuberculosis*. This approach utilizes genetically engineered reporter phage to detect viable tubercle bacilli.

Aim:

To carry out Rapid Screening of plant essential oils for antimycobacterial activity by LRP assay.

Bioassay:

Nine essential oils (calamus, camphor, cinnamon, clove, eucalyptus, lemon, lemon grass, menthe, and Tulsi oil) were selected on the basis of traditional practices, literature, survey and laboratory evaluation against routine microbes to screen for antimycobacterial activity. The reference strain *M. tuberculosis* H37Rv, was used in the screening procedure. Two concentrations of the essential oils (100 and 50µg/ml) were used for screening by LRP assay.

Result:

Cinnamon oil showed 85.99 per cent activity against *M. tuberculosis,* followed by camphor with an activity of 69.72 per cent at 100µg/ml concentration (Table 15).

Conclusion:

Cinnamon oil is a promising candidate for anti TB drug development.

Table 15: Percentage RLU reduction of essential oils against *M. tuberculosis*

Oil name	% RLU Reduction	
	50 μg/ml	100 μg/ml
Calamus oil	6.27	41.02
Camphor oil	42.04	69.72
Cinnamon oil	65.44	85.99
Clove oil	36.81	40.22
Eucalyptus oil	39.79	43.62
Lemongrass oil	40.3	48.15
Lemon oil	44.15	48.25
Mentha oil	5.3	6.48
Tulsi oil	34.75	29.38

Immune response in tuberculous pleuritis: Expression profile of toll like receptors (TLR) on various immune cells and its role in tuberculous pleuritis (TP)

Background:

Toll Like Receptors are pattern recognition receptors that are key molecules for the orchestration of cross talk among many clinical manifestation of TB. It regulates both innate and adaptive immune responses. In TP, the protective immune response is generated by both innate and adaptive immune cells at the site of infection. In our previous annual report, a differential T-helper cell response was demonstrated by intracellular cytokine studies. In this report, we studied the role of TLR in tuberculous pleuritis.

Aim:

To determine the expression profiles of TLR2 and TLR4, on various cell subsets and its impact on the Th1/Th2 outcome.

Methods:

Peripheral Blood Mononuclear Cells (PBMC) and Pleural Fluid Mononuclear Cells (PFMC) separation was done by Ficol-Hypaque density gradient centrifugation. Pure population of CD4 T lymphocytes was obtained by MACS purification. Cell-subset profiling and TLR expression was performed by flow cytometry.

Results:

CD14+ monocytes expressed maximumTLR-2 and TLR-4 in both PBMC and PFMC. TLR-2 but not TLR-4 expression was upregulated on monocytes present at the site of infection (Fig.18 & 19). Expression of TLR-2 and TLR-4 was further enhanced in IFN-γ secreting T helper cells.

Conclusion:

TLR-2 on monocytes has a prominent role at the site of infection. Higher expression of TLR-2 in IFN-γ secreting T-helper cells, suggest that it is essential for optimal mycobacterial antigen-specific TH1 cell response.

Key message:

TLR-2 mediated adaptive immune response in tuberculous pleuritis.

Fig.18: TLR-2 expression on various cells of PFMC (N=7)

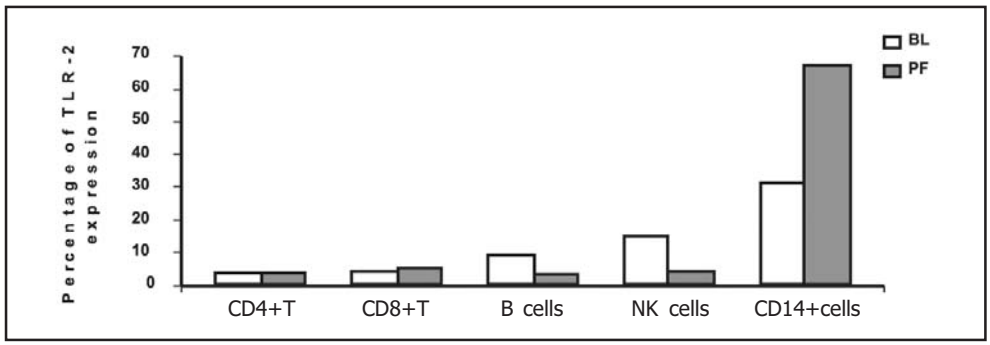
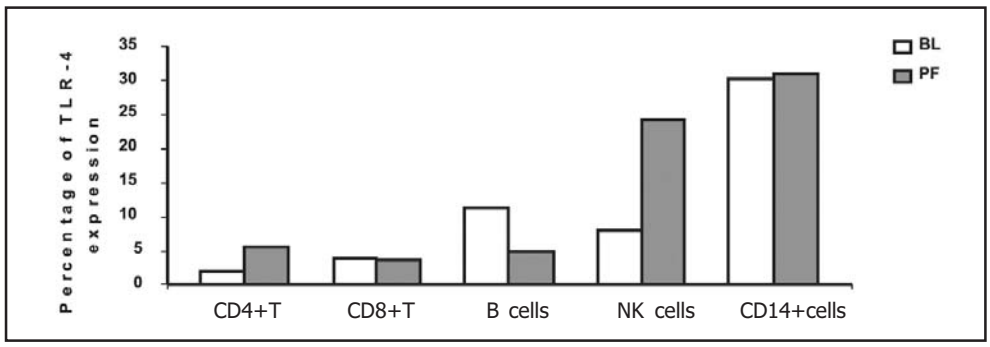


Fig. 19: TLR-4 expression on various cells of PFMC (N=7)



Molecular and Immunological characterization of *M. tuberculosis* strains with Single copy of IS6110: Modulation of immune markers and their correlation with apoptosis in THP-1 cells infected with prevalent strains of *M. tuberculosis*

Background:

The innate ability of infected macrophages to undergo programmed cell death (Apoptosis) and curtail the infection is crucial for the host defense. Although phagocytosis and intracellular killing mechanisms are highly effective in eliminating TB bacilli, some strains have evolved strategies to inhibit this microbicidal function and make use of macrophage for its successful and prolonged survival. Such modulation in macrophage functions by some *M. tuberculosis* strains is an emerging theme in pathogenesis.

Aim:

To study the induction of apoptosis in THP-1 cells infected with prevalent strains of *M. tuberculosis* and correlate with expression of CD14 and MHC Class-II molecules, TNF-α secretion, phagocytosis and colony forming units (CFU).

Materials and methods:

THP-1 cells were differentiated from the macrophages by PMA and subsequently infected with various strains of *M. tuberculosis* (H37Rv, H37Ra, prevalent and primitive clinical strains S7 & S10). The rate of apoptosis (Annexin-FITC), expression of CD14 and HLA-DR (FACS) and TNF-α (ELISA) were estimated.

Results:

The total percentage phagocytosis ranged from 60-80 in all the strains with no significance. However, based on phagocytic index (Fig.20), THP-1 cells infected with clinical strains showed low dose of infection in 1-10 bacilli category thereby exerted less burden on the cells. The uniform increase in CD14 expression was observed on all infected THP-1 cells, except H37Ra at all time points. Infection with clinical strain S7 induced less apoptosis (Fig.21) with significant decrease in HLA-DR, whereas S10 infected cells showed increased apoptosis with increase in CD14, HLA-DR and TNF-α levels at later time points.

Conclusion:

Our results indicated differential mode of infection by clinical strains which adopted the best strategy of "more spread but less burden" for their successful establishment in the host environment.

Key message:

Differential mode of infection by clinical strains and their adaptation to different survival strategies may lead to immune suppression and pathogenesis of the disease.

Fig.20: Phagocytosis of various *M. tuberculosis* strains by THP-1 cells

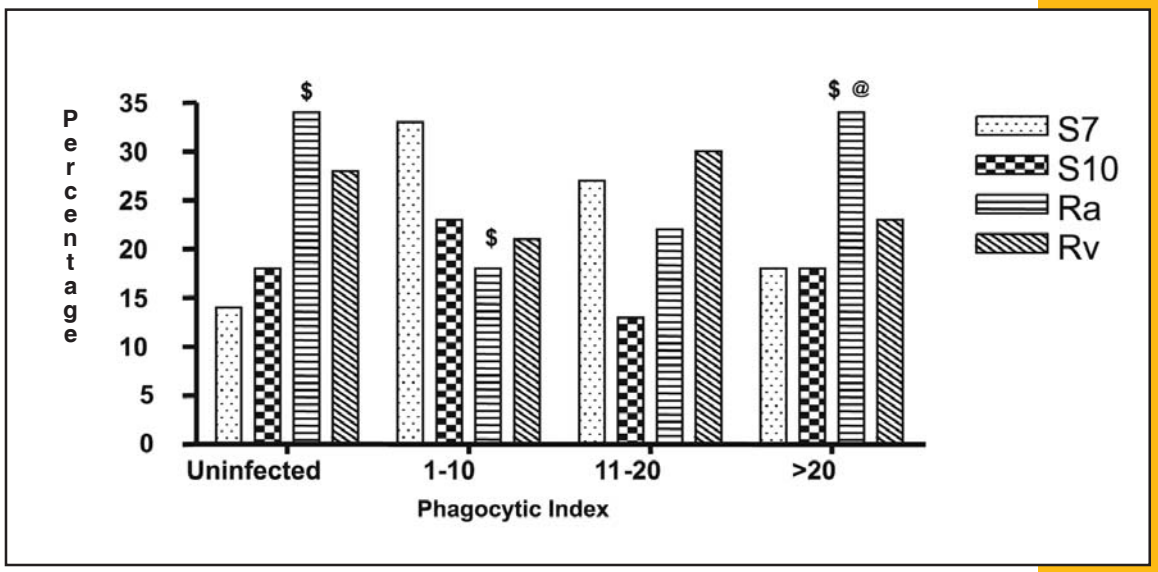
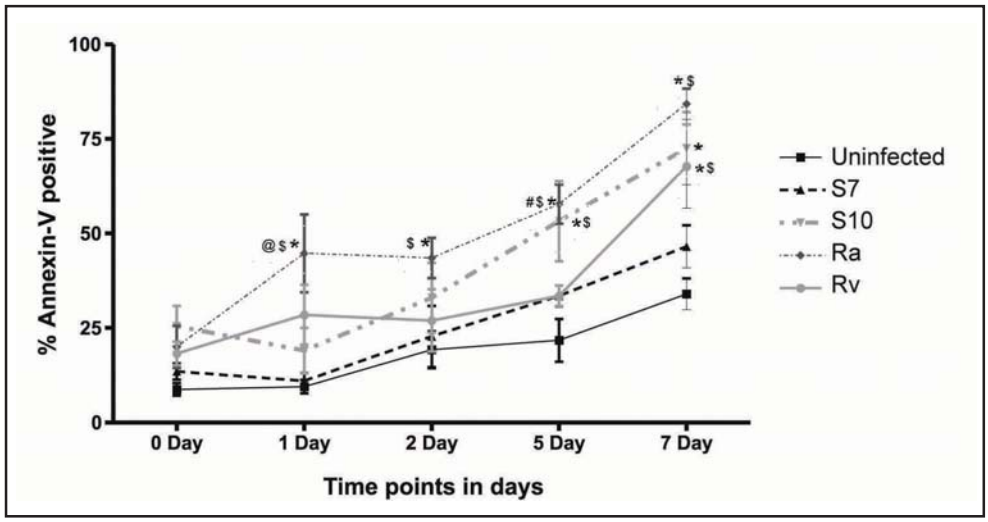


Fig. 21: Apoptosis induced by various *M. tuberculosis* strains



Role of chemokines in tuberculous Immunity: Expression profiles of chemokines and chemokine receptors on T cells in tuberculous pleurisy

Background:

Chemokines are known as the chemotactic cytokines that mediate the biological functions by binding to the specific G-protein coupled receptors exhibited on the surface of various immune cells and aid their transmigration to the site of infection. Cells of the innate immune system express various receptors for inflammatory chemokines, which is the first line of defense against the invading pathogen. Tuberculous Pleurisy is an effective model to study the role of chemokines at the site of active *M. tuberculosis* infection.

Aim:

(i) To study the expression profile of chemokine receptors on T cells of blood and pleural fluid obtained from the tuberculous pleuritis patients.

(ii) To quantify and compare the levels of chemokines and cytokines in the plasma and pleural fluid of the same patients.

Methods:

Pleural fluid and blood was collected from TB pleuritis patients.

The Pleural Fluid Cells (PF) and Blood Cells (BL) were labelled for various chemokine receptors and analyzed by Flow Cytometry.

Chemokines and cytokines were assayed by Cytometric Bead Array (CBA).

Results:

There was a significant increase in the expression levels of CCR2, CXCR2 and CXCR3 but not in CCR5 on $CD4^+$ T cells of PF compared to BL.

Similar increase of CCR1 and CCR7 was observed on $CD3^+$ T cells of PF (Fig.22 & 23). The dual function chemokines (MIG and IP-10) and inflammatory chemokines (MCP-1, IL-8 and MIP-1α) were significantly elevated in PF compared to BL. RANTES was significantly high in blood (Fig.24 & 25).

Conclusion:

Tuberculous Pleurisy is characterized by lymphocyte predominance mounting protective Th1 response. A selective concentration of chemokines, cytokines and abundant expression of chemokine receptors further confirm the accumulation of activated and memory T cells at the site of infection and help in polarizing the immune response.

Key message:

Chemokines and chemokine receptors help in migration of activated immune cells to the site of infection.

Fig. 22: Expression of α and β chemokine receptors on CD4+ T cells

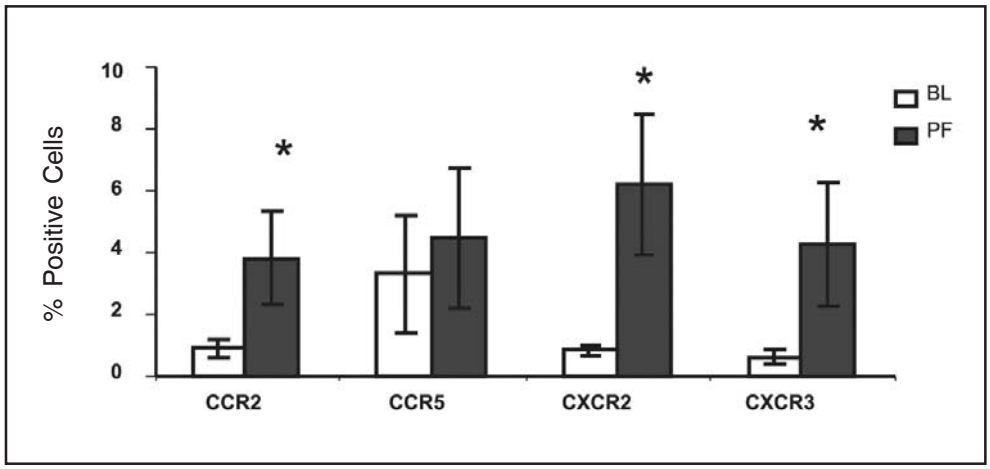


Fig. 23: Expression of β type of chemokine receptors on CD3+ T cells

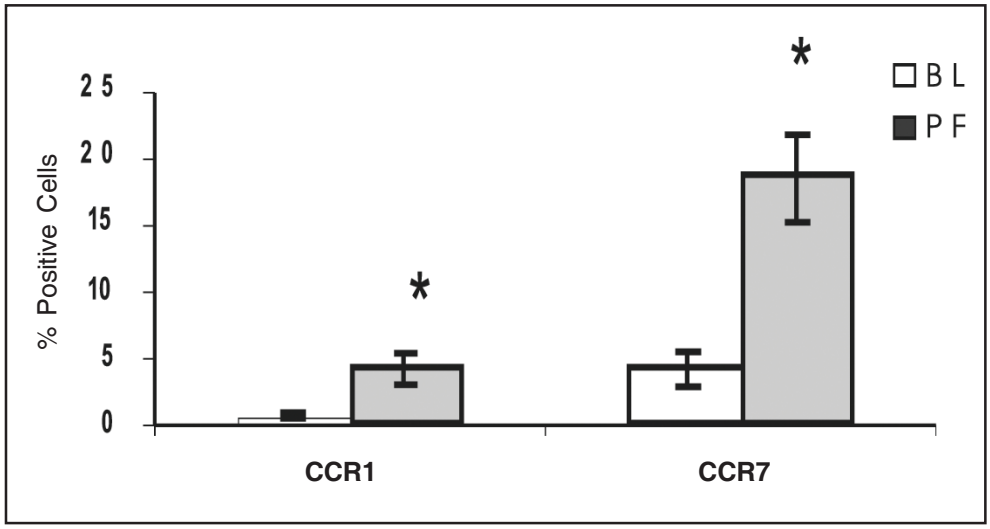


Fig. 24 : Levels of dual function chemokines in blood and pleural fluid

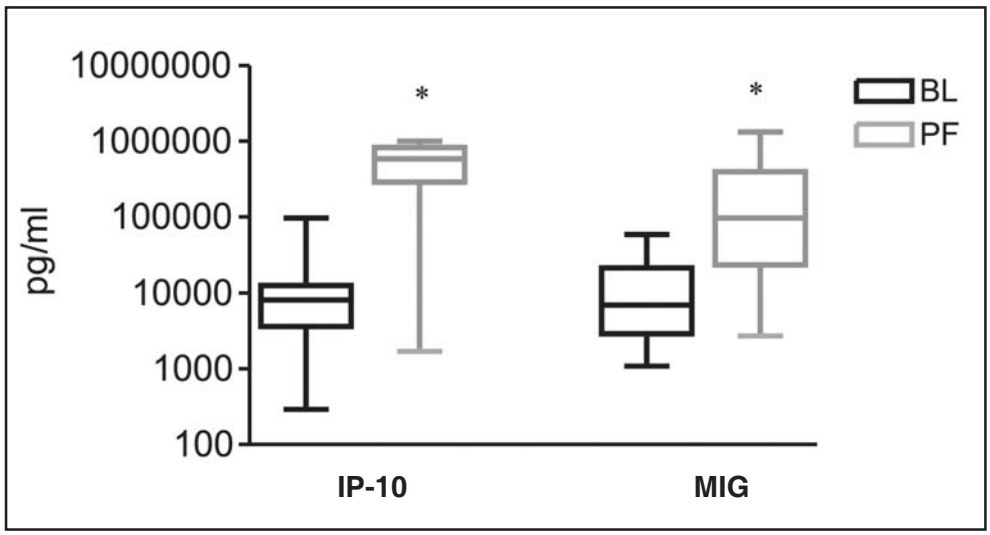
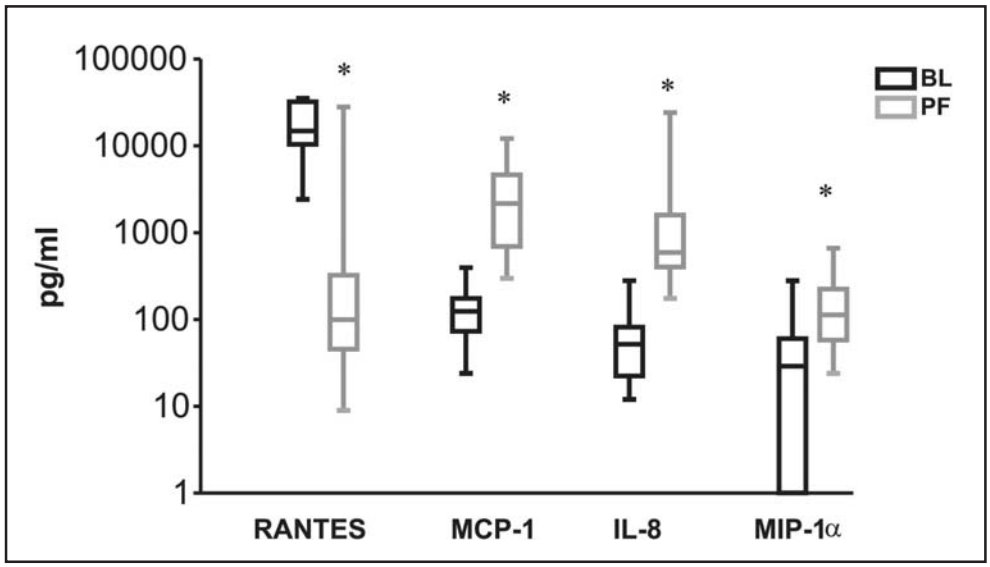


Fig. 25: Levels of inflammatory chemokines in blood and pleural fluid



Role of Dendritic cells (DC) in Mycobacterial Immunity: Differential maturation of human monocyte derived – dendritic cells by prevalent strains of *M. tuberculosis*

Background:

Dendritic cells play a key role in the pathogenesis of TB. The primary initiation and modulation of immune response is mediated through the DCs. Maturation of DC is necessary for effective priming of immune response.

M. tuberculosis has developed various mechanisms to evade host immunity. One such strategy is to inhibit the maturation of DC. It has been reported that laboratory virulent strains suppress DC maturation. Similarly the prevalent clinical strains may prove a better source for studying the association of virulence with evasion of immune mechanism by DCs.

Aim:

The study has been designed to evaluate changes in the functional and phenotypic maturation of monocyte-derived dendritic cells (MDDC) upon infection with prevalent strains (S7 and S10) and laboratory strain (H37RV) of *M. tuberculosis*.

Methods:

PBMCs were isolated from the blood of healthy volunteers. Monocytes were purified by using anti-CD14 conjugated magnetic beads. MDDC were generated by culturing $CD14^+$ cells with GM-CSF and IL-4 for six days. On day seven, the MDDC were infected at a *Multiplicity of infection* (*MOI*) of four with various mycobacterial strains. After 24 hours, DCs were harvested and analyzed using FACS for various phenotypic markers. ELISA for cytokines and chemokines was performed in the culture supernatants.

Results:

Upregulation of CD80 but not CD86, was seen after infection with Rv & S7 whereas S10 showed significant increase in CD86 (Fig.26). There was increased CD83 expression after LPS stimulation and S10 infection but not in Rv and S7 infected DCs. Down regulation of CCR5 and upregulation of CCR7 was observed in all infected DCs. The levels of IL12p-40 and TNF-α also increased after infection with all the strains. There was marginal increase in IFN-γ levels with no change in IL-1β after infection. The IP-10 levels were significantly increased in LPS and S10 whereas MIP-1á levels (Fig.27) significantly decreased in Rv infected DCs.

Fig. 26: Expression of costimulatory and maturation markers on DC

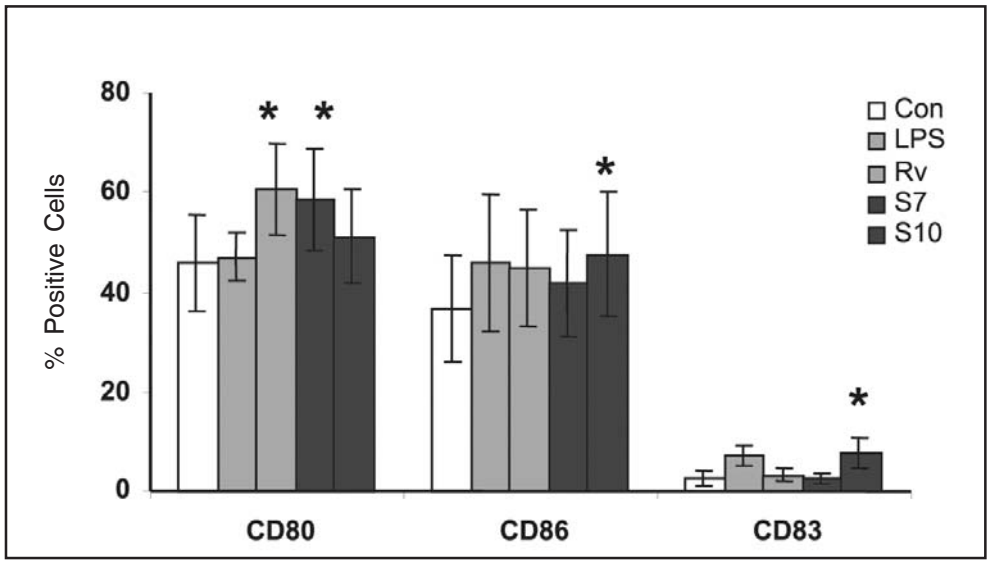
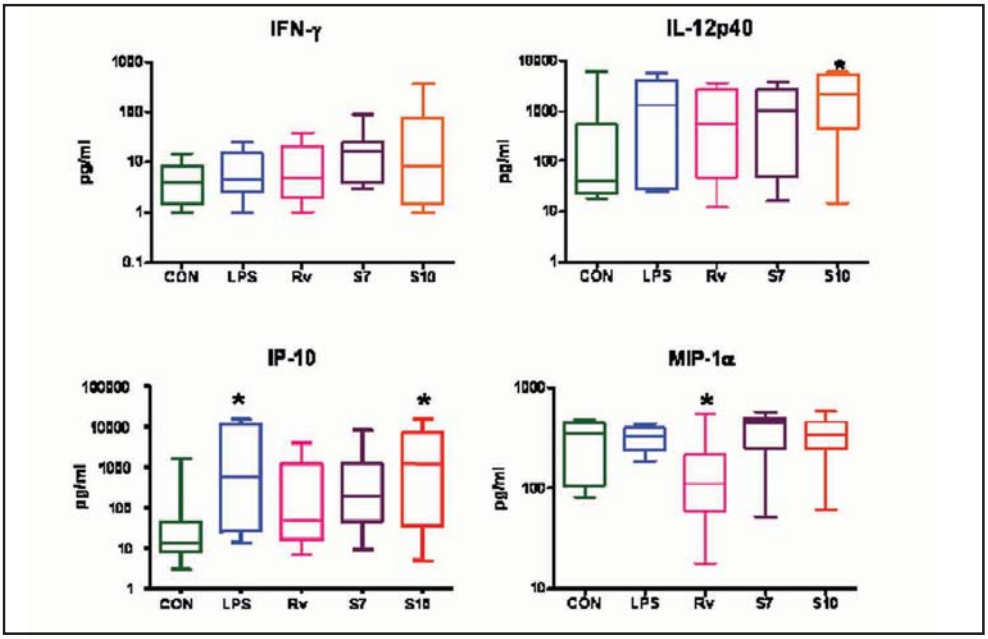


Fig. 27: Cytokine and chemokine profile of infected DCs



Conclusion:

The maturation and function of DCs are modulated by the *M. tuberculosis* strains depending upon their virulence. The laboratory strain H37Rv and clinical strain S7 are highly virulent compared to S10 in inhibiting the complete maturation of DCs. Thus the hampering of maturation of DCs is a novel mechanism adopted by *M. tuberculosis* strains to evade the effective immune response of the host.

Key message:

M. tuberculosis strains hamper DC maturation as a strategy to survive in the host.

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 tuberculosis 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 as antimycobacterial antibodies are known to be present in endemic populations, it was considered important to investigate whether antibody 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:

Phagocytosis of *M. tuberculosis* by macrophages was assessed under the following conditions:

1. Different infection ratios (macrophages:mycobacteria).
2. Different serum concentration.
3. Pre opsonization of *M. tuberculosis* with serum at different time points of incubation.

Complement receptor expressions will be assessed in PBMC using flow cytometry.

The levels of different inflammatory cytokines will be measured using flow cytometry.

Results:

The percentage of macrophages infected with heat-killed *M. tuberculosis* was lower than those with live bacilli was independent of the infection ratio used (Fig. 28). Association of *M. tuberculosis* was markedly decreased in heat inactivated pooled human serum (HI-PHS) (48.66 ± 8.17%) or under serum free condition (45.5 ± 9.04%). The ability of macrophage phagocytic index increases with increasing serum concentrations (Fig. 29). Pre opsonization of the bacilli with fresh serum also resulted in an increase in the phagocytic index (Fig. 30).

Fig. 28: Effect of infection ratio on phagocytosis of *M. tuberculosis* by macrophages (Mean ± SE)

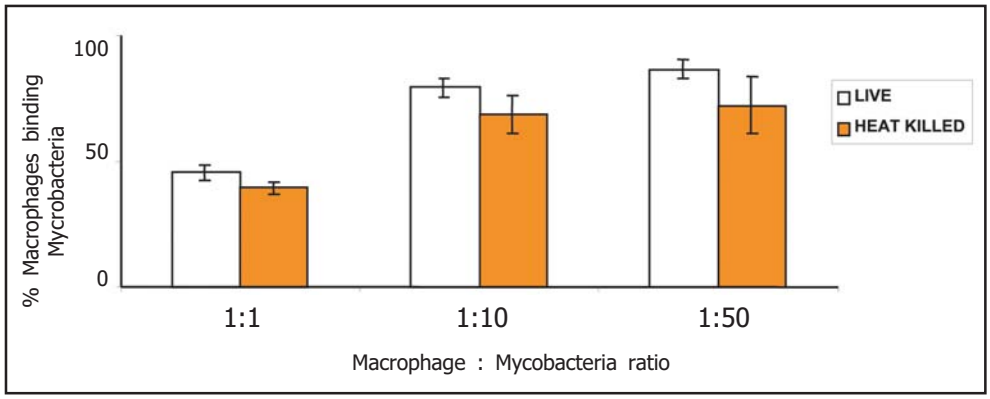


Fig. 29: Effect of oposonization (serum concentration) on phagocytosis of *M. tuberculosis* by macrophages (Mean ± SE)

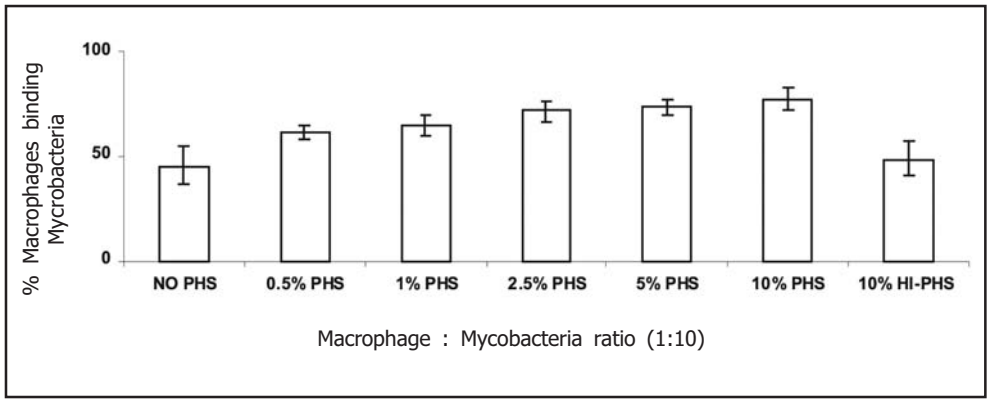
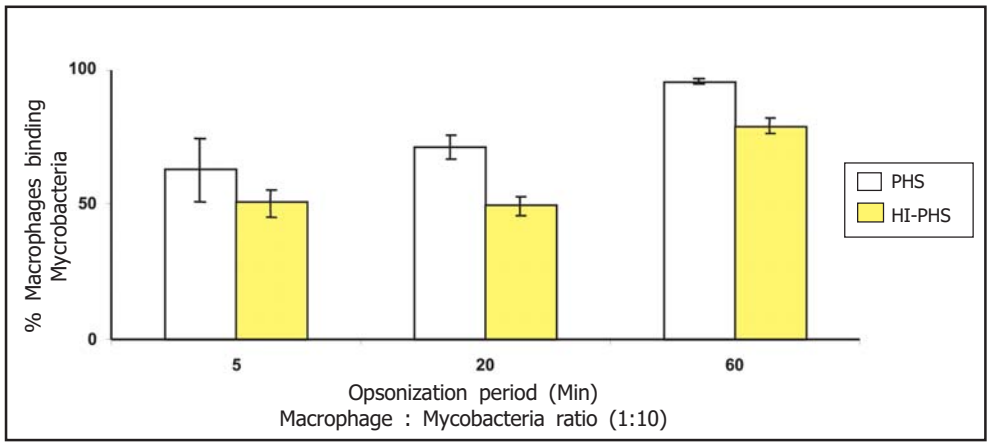


Fig. 30: Effect of opsonization (time) on phagocytosis of *M. tuberculosis* by macrophages (Mean ± SE)



The status of the complement system in tuberculosis

Background:

The complement system serves as a major link between the innate and the adaptive immune systems as well as in facilitating production of appropriate immune response to an antigenic stimulus. The following are known about the involvement of the complement system in mycobacterial infections: Mycobacteria and some of their components like PGL of *M. leprae*, cord factor and PPD of *M. tuberculosis* activate the complement system. Complement components are found in circulating immune complexes formed in active tuberculosis. Levels of complement proteins and hemolytic complement are significantly higher in patients compared to healthy controls. Mice deficient in complement components are more susceptible to *M. tuberculosis* infection than control mice.

Aim:

To investigate the status of the complement system in tuberculosis 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 tuberculosis.

Objectives:

Measurement of the levels of complement components and activation fragments in serum and complement receptors on peripheral blood lymphocytes.

Studying the alteration of the functional capacity of the activated complement system in tuberculosis.

Documenting the effect of complement on the immune responses against *M. tuberculosis.*

Participants:

The study subjects will comprise of 25 patients each with active, smear positive pulmonary tuberculosis and subjects who have completed the full anti-tuberculosis treatment regimen and 50 normal healthy volunteers.

Methods:

Complement profile in tuberculosis:

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

Functional assay:

Functional characterizations of complement activation were assessed using 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* were studied by analyzing the levels and components of circulating immune complexes using ELISA, and the effect of added complement components on phagocytosis and apoptosis of mononuclear cells using flow cytometry.

Results:

Mannose Binding Lectin (MBL) levels were found to be higher in both active and healed tuberculosis groups showing the involvement of the lectin pathway in the activation of the complement system. Also, a positive correlation was observed between MBL and C3d levels which is suggestive of the role of lectin pathway in potentiating complement system activation.

The increase in the complement activation fragments like C3d, C3a, C4a and C5a in the before treatment group reflects the disturbances in the host immune response leading to the activation of the complement system. The levels of C3 in serum from pulmonary tuberculosis patients were found to be less compared to normal healthy volunteers. This may be due to excess activation of the complement system leading to cleavage of C3 to its activation fragments.

The levels of circulating immune complexes and the presence of complement components (C3, C3d, C4c) and immunoglobulins (IgG, IgM) in the complexes were found to be elevated in active tuberculosis group compared to controls. The data obtained from flow cytometry show significantly decreased expression of complement receptors in the major subsets of lymphocytes obtained from the before treatment group compared to the treated individuals and normal controls (Fig. 31 & 32). This point to the involvement of complements receptors in the immune responses of pulmonary tuberculosis and suggests that the expression levels are modulated by the underlying disease status of the patients.

Complement admixture was found to augment apoptosis since the percentage of cells undergoing apoptosis was more in mononuclear cells incubated with serum as source of complement compared with cells incubated with heat inactivated serum (Fig.33).

Fig. 31: Expression of CR1 on B lymphocytes

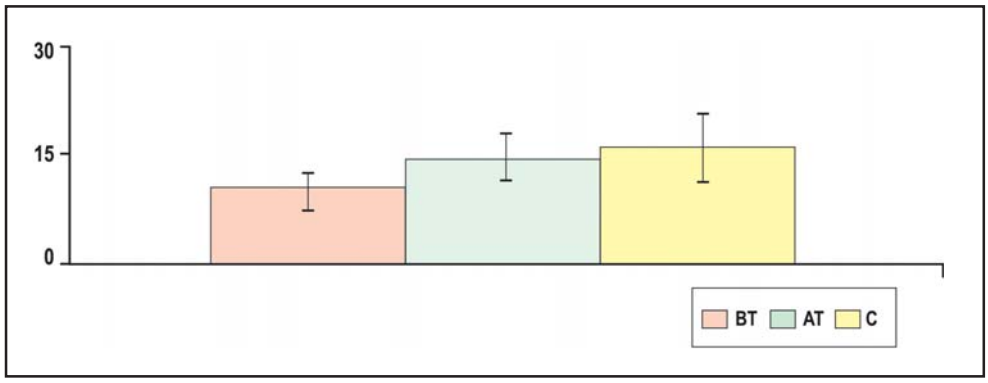


Fig. 32: Expression of CR2 on B lymphocytes

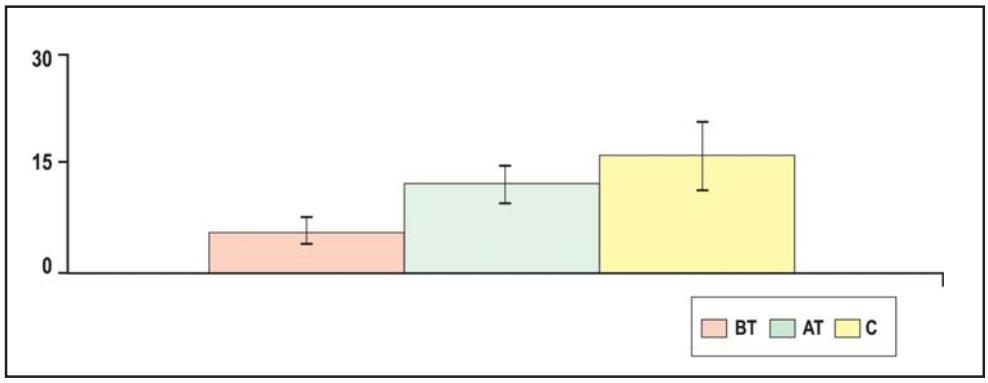
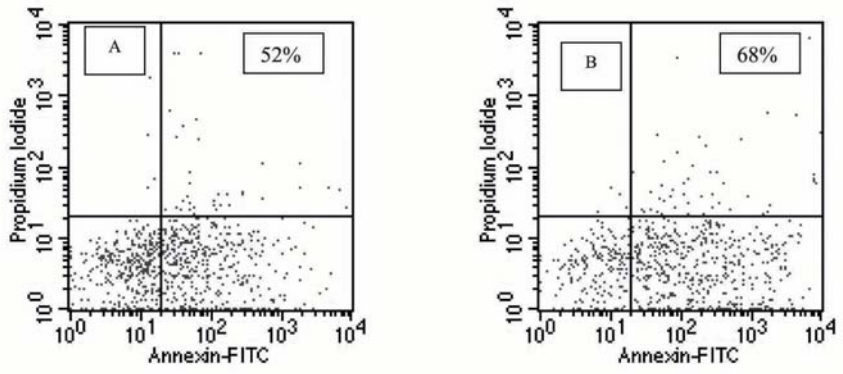


Fig.33: Augmentation of apoptosis by complement



Legend: Representative scattergram showing effect of complement on apoptosis. Apoptosis was detected by heat inactivated H37Rv-stimulated PBMC incubated with and without admixture of complement. Values indicated represent percentage of Annexin V positive cells. (A: without complement source – 52%, B: with admixture of complement – 68%).

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*.

Objectives:

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 peripheral blood leucocytes and release of various cytokines from them.

Methodology:

All the above strains are grown in Middlebrook 7H9 broth for 3-4 weeks at 37ºC. 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 the various cytokines (VNR to mention the cytokines) using ELISA.

Results:

The kinetics of the uptake of C3 and C4 by the bacilli has established that this is dependent on both the concentration of the bacilli and serum.

C3 and C4 uptake by the knock-out bacilli was found to be inversely related to number of bacilli.

Decreased C3 uptake through both pathways was observed for DKO and DevR strains compared to their respective wild strains, Erdman and H37Rv.

C4 uptake was found to be higher for all gene-disrupted strains compared to their wild strains; and inversely related to the number of organisms.

For the complemented strains, C3 uptake was found to be higher by MptpA complemented strain compared to the wild strain; VirS complemented and DevR complemented strains exhibited less values or almost equal to those of their respective wild strains (Fig. 34-36).

C4 uptake was found to be higher by MptpA complemented, VirS complemented, and DevR complemented strains, though not significant.

Fig. 34: Uptake of C3 by MptpA and the complemented strain compared to the wild type

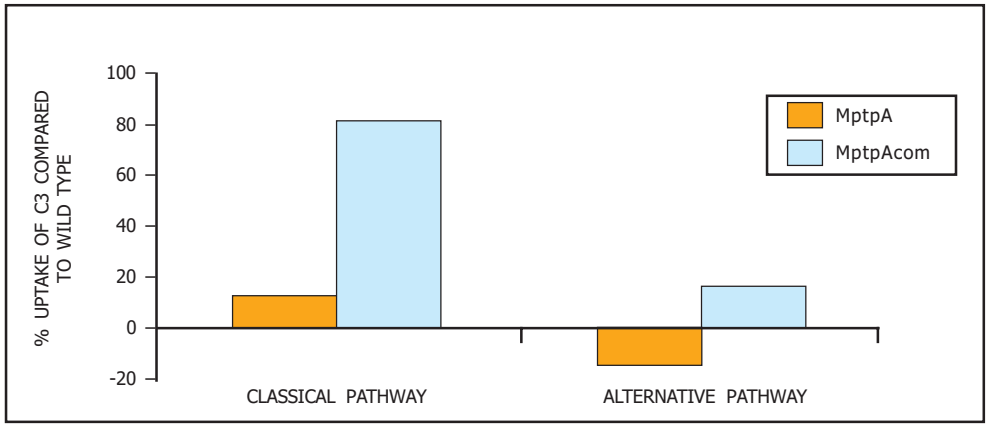


Fig. 35: Uptake of C3 by DevR and the complemented strain compared to the wild type

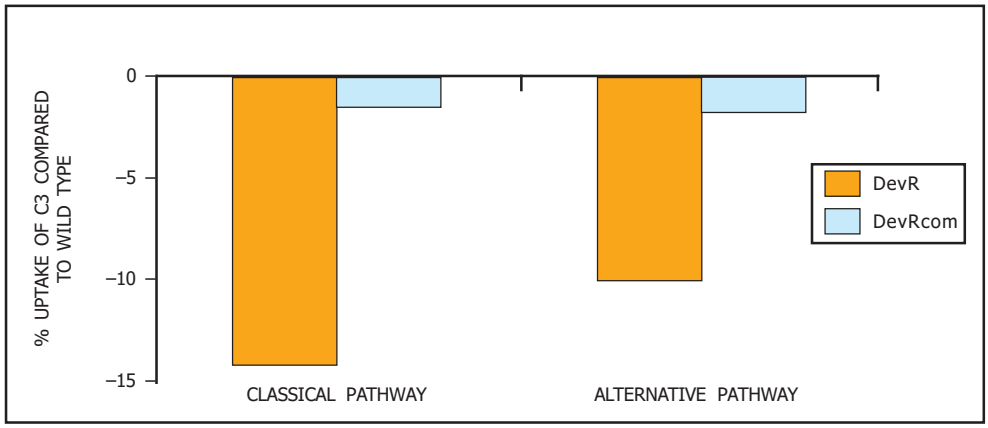
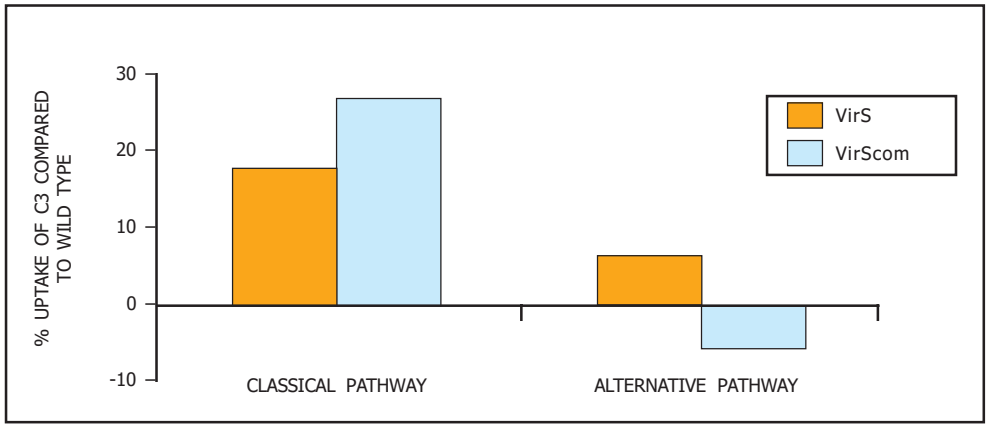


Fig. 36: Uptake of C3 by VirS and the complemented strain compared to the wild type



STATISTICAL RESEARCH

Studies Completed:

MCMC Methods for Molecular Computing

The basic principles of molecular genetics are the central dogma which states that DNA makes RNA, makes protein and those nucleic acids are sequences composed of four nucleotide subunits and that the proteins are sequences composed of 20 amino acid subunits.

The most reliable way to determine a biological molecule structure or function is by direct experimentation. However it is very difficult in most situations. It is far easier to obtain the DNA sequence of the gene corresponding to an RNA or protein. This provides a strong motivation for developing computational methods that can infer biological information from the sequence alone.

Computation methods have become especially important since the advent of genome projects. The human genome project has given us the raw sequence of an estimated 30,000 human genes, only a small fraction of which has been studied experimentally. Most of the problems in molecular computing are essentially statistical. Stochastic evolutionary forces act on genomes.

Discerning significant similarities between anciently diverged sequences amidst a chaos of random mutations, natural selections and random genetic drifts, pose serious signal to noise problems. This work discusses the use of Markov Chain Monte Carlo (MCMC) methods to provide a general structure for statistical analysis of genome data.

We often recognize a significant similarity between a new sequence and that of one about which something is already known. When we do this we can transfer information and/or function to the new sequence. We say that the two sequences are homologous and that we are transferring information by homology.

The comparison of biological sequences is similar to deciding that two text strings are similar and the extensive computer science based methods are available. However, the concept of alignment is crucial in biological sequence analysis. Evolving sequences accumulate insertion and deletions as well as substitutions. Hence, before the similarity of two or more sequences can be evaluated, one begins by finding a plausible alignment between them.

Developing more sensitive scoring schemes and evaluating the significance of alignment scores in the realm of statistics

The probabilistic matrices for scoring pair wise sequence alignment, was the first step to quantify evolutionary preferences for certain substitutions over others. Many sophisticated probabilistic modeling approaches have been brought in to computational biology by many routes. And the MCMC approach is one of the natural frameworks to address the complex inference problems in computational sequence analysis.

Many of the probability models used in molecular computing, assume that the sites on the DNA molecules are independent and identically distributed (IID) over the set of bases {A, C, G, T}. However, some patterns are available in DNA sequences. For example, purins tend to follow purins. And pyrimidines tend to follow pyrimidines. Certain sub sequences tend to occur more frequently than others and some sites are more prone to change than others. It is possible to represent a DNA sequence graphically using the following two plots.

$$\text{Let } X_i = \begin{cases} 1, & \text{if the position I is C or T.} \\ -1, & \text{if the position I is A or G.} \end{cases}$$

$$\text{Let } Y_i = \begin{cases} 1, & \text{if the position I is C or G.} \\ -1, & \text{if the position I is A or T.} \end{cases}$$

$$\text{Let } S(X_i) = \Sigma X_j, \text{ plot } S(X_i) \text{ versus I}$$

$$\text{Let } S(Y_i) = \Sigma Y_j, \text{ plot } S(Y_i) \text{ versus I}$$

The first plot looks at the preponderance of pyrimidines over purines and the second looks at the preponderance of one pair of complementary bases over other. Fig. 37 shows the plots for *M. tuberculosis* DNA sequence. We see that the assumption of IID sequence is deviated and the complementary base plot reveals less of a departure from the IID model. The adequacy of the IID model for representing long range patterns in a DNA sequence under study can be checked by comparing the above graphs calculated using the real sequence with graphs calculated using sequences simulated from the IID model. We considered a generalization of the IID model to a Markov one.

The probability of the whole sequence is determined by the conditional distribution of the next base given the preceding sequence of bases. It is assumed that this conditional distribution is determined by just a few different subsequences, which we call the effectives. If there are no effectives then the DNA is just a sequence of IID assignments of bases from the set {A, C, G, T}.

A Markov Chain is a stochastic process for which the distribution of x_i depends only on x_{i-1}. In sequence analysis, many times we want a model that generates sequences in which probability of symbol depends on the previous symbol such as promoters or start region of many genes. The simplest such model is a classical Markov Chain. A Markov Chain for DNA can be drawn as in Fig. 38.

The DNA alphabets ACGT represent four states. The probability parameter associated with each arrow in the figure determines the probability of a certain residue, following another residue or one state following another state.

The main issue in MCMC is how to construct a Markov Chain that converges to a given equilibrium distribution. The two most commonly used MCMC algorithms that are applicable to both discrete and continuous systems are:

- Metropolis Algorithms
- Gibbs Sampler

The Bayesian calculations not analytically tractable can be performed once a likelihood and prior are given. For non-Bayesian applications, MCMC is considered a very powerful numerical device in likelihood analysis or decision theory. The posterior distribution using MCMC method given in the Table 16.

Table 16: MCMC Summary Measures

Measure	τ	α
1st Quartile	19.9	3.9
Median	25.0	5.4
3rd Quartile	32.2	9.3
Mean	28.4	6.9

Fig.37: gatc-tagc plots

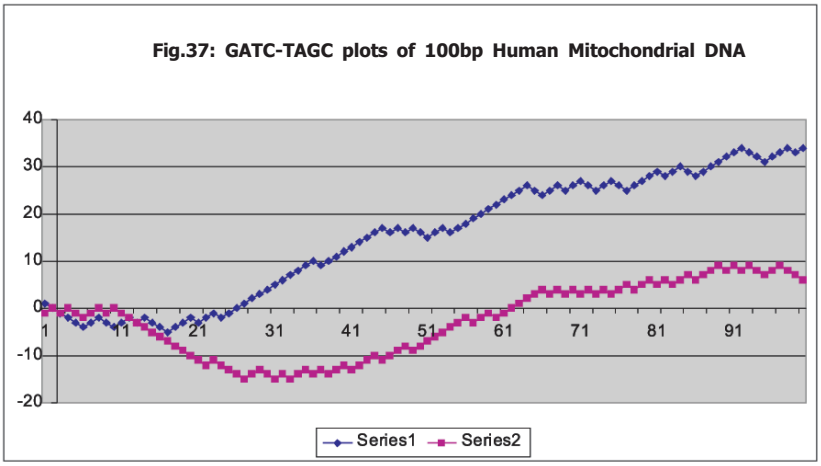
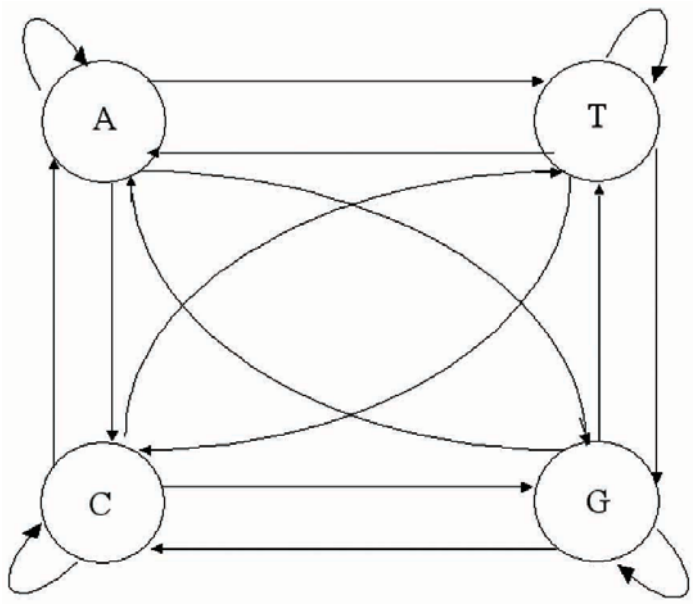


Fig.38: Four state Markov model for DNA



Studies ongoing:

Machine Learning Research

The linear description of protein, DNA and RNA molecules has changed the character of computational analysis of biological sequences.

Computational tools for classifying sequences, detecting weak similarities, separating protein coding regions from non-coding regions in DNA sequence, prediction molecular structure and function, and reconstructing the underlying evolution history, are the major focus of current research. This is essential to our understanding of life and evolution as well as to the discovery of new drugs and therapies.

Large databases of biological information create both challenging data mining problems and opportunities, each requiring new ideas. Conventional computer science algorithms have been useful, but are unable to address many of the interesting sequence analysis problems.

This is due to the inherent complexity of biological systems, brought about by evolutions' tinkering and our lack of a comprehensive theory of life's organization at the molecular level. Machine learning approaches, such as artificial neural networks, support vector machines, genetic algorithms and related techniques like hidden Markov models, Markov Chain Monte Carlo and belief networks, are ideally suited for domains characterized by the presence of large volume of data, noisy patterns and the absence of general theories.

The fundamental idea behind those approaches is to learn the theory automatically from data, through a process of inference, model fitting and learning from examples. The nature of biological sequence data, has a profound impact on the types of algorithms which have been developed and applied for computational analysis. While the goal often is to study a particular sequence and its molecular structure and function, the analysis typically proceeds through the study of an ensemble of sequences consisting of its different versions in the same species.

Machine learning techniques form a viable complementary approach to conventional molecular computing methods. Research on various machine learning approaches are currently in progress.

LIBRARY AND INFORMATION SERVICES

The library services gather, maintain and make accessible anything to do with health information.

This Library has tremendous campus wide electronic access facility for information services. A remarkable progress has been established with the wealth of electronic resources. It caters to the need of scientists' demand on time. The refurbished modular library provides beautiful learning ambience.

Internet Browsing Lab:

The high bandwidth Internet connectivity at the browsing lab is being effectively utilized by Staff of TRC/ICMR, project staff and students from outside.

Renovated Library

Internet Browsing Lab

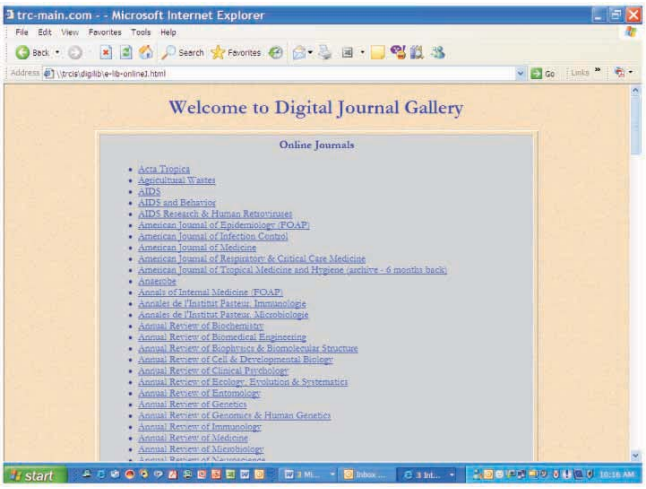
Value Added Services:

Digital Library:

The TRC library is trying to get as many resources as possible in one location, viz, Digital Library.

It serves as a web-based interface to the in-house resources and hyperlink to all the full-text e-journals; e-databases; e-subject collection and publisher's cumulative collections are being subscribed. ICMR's resource sharing electronic databases viz., ProQuest; OVID; J-gate and J-Content Consortia and Web OPAC have further strengthened the digital library platform.

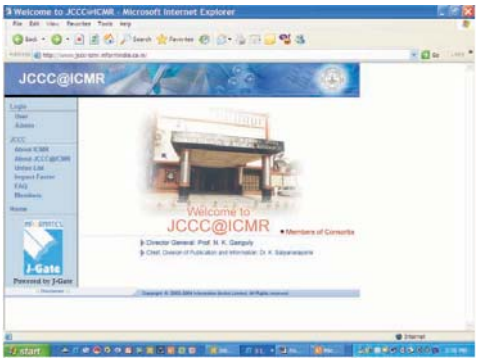
Library services' forms are made available to the user community through digital library. Bibliographical details of TRC Publications; Periodicals Holding list of library and Instructions to authors are also made available electronically. The main objective of this platform is to save time.



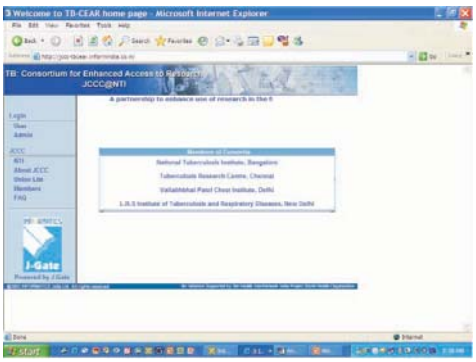
JCCC:

The Journal Custom Content Consortia has been actively fulfilled by joining hands with Indian Council of Medical Research at JCCC@ICMR; and four major TB institutes in India at JCCC@HIN under the World Health Organization's Health InterNet work Project.

JCCC@ICMR



JCCC@HIN



The SDI services are being done through Internet and CD-ROMs.

Open Access Resources:

Under the digital library umbrella, there are the pointers to outside online resources through the Open Access Resources— viz., Dictionary, Encyclopedia, Thesaurus, Search Engines, TB link, medIND, J-Gate, Specialized Health Science Databases etc. The digital library tries to make it easier to access available resources from various countries in the world.

Resource Sharing:

The Institute sustains its institutional membership facility at the British Council Library, Chennai.

The library actively participated in the ICMR librarians group and shares the existing resources on demand expeditiously.

Electronic databases, ProQuest; OVID and the consortia JCCC@ICMR; and JCCC@HIN are the best platforms to share the resources among ICMR institutes and all the four major TB institutes in India.

Multimedia Unit:

The Multimedia Unit is doing wonderful service in the area of conversion of print document to the electronic format with scanning and editing business; annual report co-ordination for designing and compilation; organizing and managing distinguished visitors/conferences/workshops' photographs; co-ordination for scientific publications and thesis work relating to scanning and editing; Portable Document Format (PDF) conversion for print article; Geographical Information System analysis; co-ordination for designing and data management for TRC Web site; and poster designing for scientists.

Future plan:

To introduce 2D and 3D animation designing for scientific related works and video/ audio mixing and editing.

Automation:

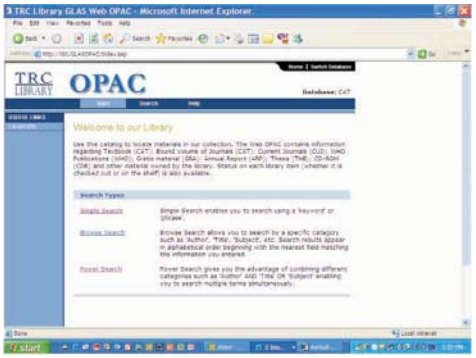
The Library automation process is being progressively followed through the software GLAS (Graphical Library Automation System, USA) to integrate circulation and collection control.

There is also a plan to introduce e-mail interaction system.

OPAC:

The Online Public Access Catalog of TRC library is being made available through GLAS Software.

The interface to access the current status of the material(s) can be done with this software through digital library.



E / print Collection development:

The electronic collection development falls on e-journals, e-databases, individual titles, cumulative collection etc. It covers nearly 89 per cent of the library's subscription.

ELECTRONIC DATA PROCESSING

The Electronic Data Processing (EDP) division provides computerized services to 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 has also undertaken data entry process for the mortality survey conducted in Orissa and Andhra Pradesh.

Data entry training was provided to a staff of RMRC, Jabalpur during the year. The recruitment of four new data entry operators got underway exclusively for this survey.

Data entry, information process and e-mailing are the key requirements for our research organization. These require a very secure and robust infrastructure. Much of the focus of the past year has been directed towards increasing the capacity of the existing IT infrastructure.

Significant developments during the year include the up-gradation of network servers, completion of the implementation of the new e-mail system of the staff, provision of a projector in each conference room and provision of a fast bandwidth link to the user desktop. Replacement and completion of the entire network cabling in TRC has been undertaken. This includes the local hubs with switches and fiber uplinks to provide users with high bandwidth connection.

The transfer of all staff e-mail accounts to the new e-mail system was completed during the year. The implementation of the Exchange e-mail system was completely re-organized. Anti-virus software was kept up to date at all times and checking all incoming e-mail for viruses.

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, six data entry/verification operators, six 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, 2005 to March, 2006 is shown below.

No. of documents entered: 3, 02,361

No. of documents verified: 3, 22,611

A total of 1, 50,786 records were processed for the on-going second resurvey of disease survey conducted at Tiruvallur district.

Twenty three panchayats' pre-printed cards, and 19 panchayats' person's alphabetical name-wise and household-wise lists were supplied for the survey. The second resurvey will be completed by June, 2006 and third resurvey will be started from July, 2006.

APPENDICES

Publications

Publications in	I) International journals	:	34
	ii) National journals	:	24
Accepted for publications in	I) International journals	:	11
	ii) National journals	:	9

Published

International:

1. Ramachandran G, Hemanth Kumar AK, Gurumurthy P, Rajasekaran S, Padmapriyadarsini C, Bhagavathy S, Venkatesan P, Sekar L, Swaminathan S. Effect of HIV on pharmacokinetics of antituberculosis drugs. Contagion. 2005; 2(2):182-83.
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3 Sulochana S, Rahman F, Paramsivan CN. *In vitro* activity of fluoroquinolones against *Mycobacterium tuberculosis*. J Chemother. 2005;17(2):169-73.
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15. Swaminathan S, Paramasivan CN, Ponnuraja C, Iliayas S, Rajasekaran S, Narayanan PR. Anti-tuberculosis drug resistance in patients with HIV and tuberculosis in South India. Int J Tuberc Lung Dis. 2005;9(8):896-900.

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1. Charles N, Sudha G, Santha T, Ponnuraja C, Fathima R, Chandrasekaran V, Jaggarajamma K, Thomas A, Gopi PG, Narayanan PR. Evaluation of directly observed treatment providers in the Revised National Tuberculosis Control Programme. Indian J Tuberc. 2005;52:73-77.
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Papers accepted for publication

International

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2. Ramachandran G, Kumar HK, Swaminathan S, Venkatesan P, Kumaraswami V, Greenblatt DJ. Simple and rapid liquid chromatography method for determination of efavirenz in plasma. J Chromatogr B Analyt Technol Biomed Life Sci.
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5. Senthamaraikannan K, Vallinayagam V, Venkatesan P. Molecular Computing using Markov Chain Monte Carlo methods. Int J Signal Processing.
6. Santha T, Subramani R, Gopi PG, Chandrasekaran V, Narayanan PR. Status of bacillary tuberculosis cases identified in a community survey under different control programmes. Inter J Tuberc Lung Dis.
7. Kumar V, Balaji S, Gomathi NS, Venkatesan P, Jayasankar K, Prabakaran L, Narayanan PR. Phagebiotics to control the exponential growth of normal flora in processed sputum specimens grown overnight in liquid cnfmedium for rapid TB diagnosis. J Clin Microbiol.
8. Selvaraj P, Jawahar MS, Rajeswari R, Alagarasu K, Vidyarani M, Narayanan PR. Role of mannose binding lectin gene variants on its protein levels and macrophage phagocytosis with live *Mycobacterium tuberculosis* in pulmonary tuberculosis. FEMS Immunol Med Microbiol.
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11. Selvaraj P, Swaminathan S, Alagarasu K, Raghavan S, Narendran G, Narayanan PR. Association of HLA-A11 with resistance and –B40 and –DR2 with susceptibility to HIV-1 infection in south India. J Acq Immune Defic Syndrome.

National

1. Selvaraj P, Prabhu Anand S, Jawahar MS, Chandra G, Banurekha B, Narayanan PR. Promoter polymorphism of IL-8 gene and IL-8 production in pulmonary tuberculosis. Curr Sci.

2. Joseph P, Chandrasekaran V, Thomas A, Gopi PG, Rajeswari R, Balasubramanian R, Subramani R, Selvakumar N, Santha T, Narayanan PR. Influence of drug susceptibility on treatment outcome and susceptibility profile of 'failures' to Category II regimen: a report from a rural area in south India. Indian J Tuberc.
3. Gopi PG, Subramani R, Santha T, Radhakrishnan S, Chandrasekaran V, Rajeswari R, Balasubramanian R, Thomas A, Muniyandi M, Narayanan PR. Performance of a DOTS programme: administrative and technical challenges a field report from a district in south India. Indian J Tuberc.
4. Selvakumar N, Ravikumar D, Sivagamasundari S, Gopi PG, Narayanan PR. A novel method of staining acid-fast bacilli (AFB) in sputum containers. Indian J Med Res.
5. Nisha Rajeswari D, Selvaraj P, Jawahar MS, Adhilakshmi AR, Vidyarani M, Narayanan PR. Elevated percentage of perforin positive cells in active pulmonary tuberculosis. Indian J Med Res.
6. Selvakumar N, Ravikuma D, Sivagamasundari S, Gopi PG, Narayanan PR. A novel method of staining acid-fast bacilli (AFB) in sputum containers. Indian J Med Res.
7. Selvakumar N, Sekar G, Kumar V, Rao DVB, Rahman F, Narayanan PR. Centrifuged deposit smears of sputum samples transported in Cetyl Pyridinium Chloride for AFB microscopy. Indian J Med Res.
8. Hemanth Kumar AK, Ramachandran G, Sharadha B, Narendran G, Swaminathan S. Urine nevirapine is not an useful predictor of antiretroviral adherence. Indian J Med Res.
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Book

1. Rani Balasubramanian, Rajeswari Ramachandran. "Evolution of chemotherapeuutic regimens in the treatment of tuberculosis and their scientific rationale" In "Tuberculosis" (Ed S.K. Sharma). 2005.
2. Fraser Wares, Rani Balasubramanian, Sharma SK, Mohan A. "Extra-pulmonary tuberculosis; Management and control ". In "TB control in India" (Eds: SP Agarwal and LS Chauhan) 2005; 95-114.
3. Santha Devi T, Rajeswari Ramachandran. The role of the National institutes In Tuberculosis Control. In "TB Control in India". (Ed. SP Agarwal, LS Chauhan) 2005.
4. Rajeswari Ramachandran, Adhilakshmi AR, Banu Rekha VV, Narendran G, Rani Balasubramanian. Extra pulmonary TB in the HIV Era. In "Practical Approach to TB management" (Ed. V K Arora) Jaypee Brothers 2005; 87- 98.
5. Rani Balasubramanian, Rajeswari Ramachandran. Clinical issues in TB meningitis. In "Practical Approach to TB Management" (Ed. VK Arora) Jaypee Brothers, 2005; 172-190.
6. Rajeswari Ramachandran, Adhilakshmi AR, Banu Rekha VV, Narendran G, Rani Balasubramanian. Latent TB infection (Dormancy) and treatment. In "Practical Approach to TB management" (Ed. V.K Arora) Jaypee Brothers 2005; 404-13.

Participation in Conferences / Seminars / Workshops/Training programmes

The centre has provided opportunities to its staff members (research and technical) and students for their professional development through participation in workshops, conferences, seminars and training programmes at national and international level. The summary of national and international level participation is given below:

At national level:

Conferences	–	37
Seminars	–	20
Workshops	–	17

At international level:

Conferences	–	4

Special Assignments

Dr. Rajeswari Ramachandran

Member of the DOTs plus committee constituted by CTD for evolving policies to treat MDR tb patients – 2005-2006.

Dr. Rani Balasubramanian

Nominated as the member of National Task Force constituted for involving all the Medical colleges in the country in RNTCP.

Dr. M.S. Jawahar

- Member of DOTS Plus Committee constituted by the Central TB Division of the Ministry of Health, Government of India to formulate guidelines for the DOTS Plus Programme.
- Member of the DOTS Plus Writing Committee constituted by the Central TB Division of the Ministry of Health, Government of India, to produce written documents for the guidelines.

Dr. Rema Mathew

- Founder Member of the Forum for Ethics Review Committees in India (FERCI)
- On the Panel of Reviewers of manuscripts for Indian Journal of Medical Research (IJMR)

Dr. N. Selvakumar

- Panel Member of 39th Annual Conference of Indian Pharmacological Society. Chennai. - Issues in management of HIV and TB infection. Dec. 28, 2005.

Membership in Committees, Consultancy:

- WHO RNTCP consultants review meeting, Suraj Kund, 28-30 March, 2005. RNTCP - Central TB Division Laboratory Experts meeting. New Delhi, 9-10 Sep, 2005.
- WHO Short Term Consultant. Monitoring visit to ICCDDRB Dakha, Bangladesh, 10-11 May, 2005.
- WHO Short Term Consultant. EQA protocol development DPRK, 31.5.05-13.6.05.
- IUATLD - International Consultants Meeting for EQA of sputum AFB microscopy, Belgium, - 1-4 August, 2005.
- IUATLD Regional Advisor Tirupura State strengthening on EQA of sputum microscopy. 5-12 February, 2006.

Dr.V.D. Ramanathan

Histopathology Consultant for Chingleput Leprosy Teaching and Research Institute.

Dr. P.Venkatesan

- General Secretary- Indian Society for Medical Statistics.
- Member- Editorial Board: Journal of Pure and Applied Spectrophysics.
- Chairman- Board of Studies-MSc (Bioinformatics)-Sri Ramachandra Medical College & Research Institute (Deemed University) Chennai.
- Member- Board of Studies-M.Sc (Human Genetics) & B.Sc (Biomedical Sciences) - Sri Ramachandra Medical College & Research Institute (Deemed University) Chennai.
- External examiner for evaluation of PhD theses for the award of PhD Degree from Karnatak University, Dharwar and National Institute of Mental Health and Neurosciences (Deemed University), Bangalore.
- Examiner to conduct the Practical Examination and evaluate the theory paper & Dissertation for M.Sc., Bioinformatics, Human Geneics at Sri Ramachandra Univeristy, Chennai.

Dr. Alamelu Raja

- Expert Member of the Institutional Review Board of Sri Kanchi Kamakoti CHILDS Trust Hospital.
- Member, Editorial Board, Indian Journal of Medical Research.
- Reviewed Ph. D Thesis submitted to Universities.
- Sree Chitra Tirunal Institute for Medical Sciences & Technology, Trivandrum
- The Tamil Nadu Dr. MGR Medical University, Chennai.
- Examiner to review the work done for a Ph. D thesis for a Senior Research Fellow at Sri Ramachandra University, Chennai.
- Examiner and member of the committee for up gradation of CSIR fellowship for Ph. D students.
- Member of the panel of examiners in Nagpur University, Faculty of Medicine.
- Examiner to conduct the Practical Examination and evaluate the theory paper & Dissertation for I M. Sc Biotechnology students at Sri Ramachandra University, Chennai.

Dr. Sujatha Narayanan

- Board Member of Meenakshi College for Women, Chennai.
- Examiner for Ph.D. thesis.
- Examiner to review the Ph.D. thesis of a student from Indian Institute of Science, Bangalore.
- Examiner to review the Ph.D. thesis and conduct viva voce examination of a student from Bangalore University.
- Member of the committee for upgradation of ICMR & CSIR fellowship for Ph.D. students.

- Member of the committee for up gradation of ICMR & CSIR Fellowship for Ph.D students.
- Examiner to conduct the Biotechnology practical examination for II M.Sc. Bioinformatics students at Stella Maris College, Chennai.

Dr. P. Selvaraj

- Executive Council Member, Indian Society for Histocompatibility and Immunogenetics, New Delhi.
- Reviewer for the Journals - Am. Rev. Resp. Crit. Care & Medicine and Journal of Clinical Immunology.
- External Examiner for M.Sc., Theory and Practical exams for M.Sc., (Human Genetics) & M.Sc., (Biotechnology) at Sri Ramachandra University, Chennai.

Staff development programme such as higher studies, training programmes, etc.

Dr.P. Selvaraj was awarded three month fellowship from 1st October to 31st December, 2005 was provided by NIH, USA, under NIAID/TRC/ICER programme to visit Dr. Steven M. Holland's Laboratory, NIAID, NIH, USA, to establish collaborative research with Dr. Steven M. Holland and Tuberculosis Research Centre.

Mrs. Sulochana Somasundaram was awarded Ph.D. degree in Microbiology by the Univesity of Madras, in November, 2005.

Ph.D. Scholars

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

Sl.No.	Name of the candidate	Month	Title of the Ph.D. degree	Supervisor/Guide
1.	Ms.G. Chandra	30.06.2005	Studies on the influence of vitamin D_3, and the polymorphic variants of vitamin D receptor gene on the immune functions to M. tuberculosis antigens in pulmonary tuberculosis	Dr. P. Selvaraj
2.	Mr.V. Aravindhan	08.11.2005	Construction of recombinant BCG based HIV-1 PND epitope delivery system	Dr.P.R. 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 on
1.	Mr. Deepak Jayakumar	Cloexp Cloning, expression and characterization of a serine threonine protein kinase, *PknE* of *M. tuberculosis* H37Rv	Dr. Sujatha Narayanan	December, 2005
2.	Ms.G. Radha	Cloning, expression and characterization of a serine threonine protein kinase, *PknI* of *M. tuberculosis* H37Rv	Dr. Sujatha Narayanan	January, 2006

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. degree	Supervisor/Guide
1	2	3	4	5
1.	Ms. R. Priya	ICMR	Apoptosis of human monocytes and macrophages by *M. tuberculosis* and its implications on cell mediated immune response	Dr. Sulochana Das
2.	Ms. Nisha Rajeswari	ICMR	Influence of HLA-DR antigens on immune functions in pulmonary tuberculosis	Dr. P. Selvaraj
3.	Ms. C. Prabha	ICMR	Immune response in tuberculosis: TH1/TH2 paradigm	Dr. Sulochana Das
4.	Ms. M. Vidya Rani	ICMR	Regulatory role of vitamin D receptor gene polymorphism on cytokine response in pulmonary tuberculosis	Dr. P. Selvaraj
5.	Ms. G. Shenbagavalli	ICMR	Serum and tissue complement profile in tuberculosis	Dr. V.D. Ramanathan
6.	Dr. P.L. Natarajan	CSIR	Cellular immunology of TB and HIV/TB	Dr. Sujatha Narayanan

Sl.No.	Name of the candidate	Source of Funding	Title of the Ph.D. degree	Supervisor/Guide
1	2	3	4	5
7.	Mr. D. Anbarasu	CSIR	Identification and characterization of immunoreactive T-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
8.	Mr. S. Manivannan	CSIR	The role of complement activation and antibody in the early interaction of *M. tuberculosis* and macrophages	Dr. V.D. Ramanathan
9.	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
10.	Mr. Ramana Rao, P.V.	ICMR	Innate immunity in HIV infection	Dr. Alamelu Raja
11.	Mr. Madhan Kumar, M.	CSIR	Cyototoxic cellular response in tuberculosis	Dr. Alamelu Raja
12.	Ms. P. Supriya	ICMR	Role of chemokines in tuberculous immunity	Dr. Sulochana Das
13.	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
14.	Mr.S. Raghavan	ICMR	Human Leucocycte Antigen (HLA) polymorphism studies in HIV and HIV-TB patients	Dr. P. Selvaraj
15.	Ms. Harini Laxminarayan	UGC	Study on Molecular Biology of *Mycobacterium tuberculosis*	Dr. Sujatha Narayanan
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.	Ms. Aparna J Christy	ICMR	Development of epitope delivery system for construction of recombinant BCG vaccine for tuberculosis	Dr. Sujatha Narayanan
18.	Ms.P. Rajashree	ICMR	Role of Dendritic cells in tuberculous immunity	Dr. Sulochana Das
19.	Mr.S. Basirudeen	ICMR	Interferon gamma assay for latent TB infection in HIV patients	Dr. Alamelu Raja
20.	Ms.S. Priya	ICMR	A study of natural killer cells in HIV-TB co-infected patients during and after treatment	Dr. Alamelu Raja
21.	Ms.A. Nusrath Unissa	ICMR	Microbiology and Bio-technological aspects of drug resistance and action in tuberculosis	Dr. N. Selvakumar
22.	Ms.S. Lakshmi		HIV drug resistance	Dr. P.R. Narayanan
23.	Mr. Kaustuv Nayak		Evaluation of cellular immune response to infection with HIV–I C subtype in South India	Dr. P.R. Narayanan
24.	Ms.N.R. Yamuna		Classification and regression trees	Dr. P. Venkatesan

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

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.	Ms. Gomathy Sekar	Optimizing sputum microscopy to detect AFB	Dr. N. Selvakumar
3.	Ms. N.S. Gomathy	Development of rapid methods for diagnosis and drug susceptibility testing of *M. tuberculosis*	Dr. Vanaja Kumar
4.	Ms. Nalini Sundar Mohan	Measurement of drug resistance in tuberculosis	Dr. C.N. Paramasivan
5.	Ms. Mohanarani Suhadev	Sociological aspects of HIV/AIDS	Dr. Udaya Mahadevan
6.	Ms. Silambu Chelvi	Antimycobacterial bioactive compounds from marine actinomycetes	Dr. Vanaja Kumar
7.	Mr. C. Ponnuraja	Frailty models	Dr. P. Venkatesan
8.	Mr. N. Arunkumar	Modelling of HIV/AIDS Epidemics	Dr. P. Venkatesan
9.	Mr. V.N. Azger Dusthackeeer	Mycobacterial latency and tuberculosis diagnosis	Dr. Vanaja Kumar

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PGCDM., D.S.Q.C.O.R.(ISI),S.D.S.(ISI)
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C.C.B. Sathya Narayanan
S. Mookkan
K. Chandran
S. Sundararajan

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Munusamy, K.
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Ishwori Dhakal
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A. Pratap Singh
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P. Madhan Kumar
S. Innamuthan

Scout

J. Jeeva
F. Albert
N. Srinivasan
K. Vasudevan
R. Purushothman
S. Prakasam

Sr. Helper

P. Ellamanda

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50
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