TUBERCULOSIS RESEARCH CENTRE

Research Activities

April 2008 – March 2009

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Preface

This edition of the Annual Report of the Centre highlights the efforts of the Centre to provide high quality research support for tuberculosis (TB) control not only in India but in other parts of the world as well through linkages with global organizations. During the year under review, our efforts to evolve patient friendly and cost effective regimens for the treatment of TB continued. This included studies to shorten the duration of treatment regimens for TB, development of regimens for people with HIV and TB dual infections and design of regimens for the treatment of MDR-TB. As before, the Centre worked closely with the TB control programme in identifying and answering key operational research questions to strengthen its efforts to control TB. The studies carried out by the Epidemiology Unit of the Centre have been widely recognized and the protocols developed by the Centre to assess the ARTI have now been adopted and are currently being utilized in other locations by the Central TB Division. Major laboratory activities included studies on the immunology and molecular biology of TB and immuno-genetics of TB. The Centre also provides valuable statistical support to investigators all over India and plays a lead role in modeling and projection exercises conducted by national and international agencies.

The high quality research output of the Centre is due to the untiring efforts of all the scientific, technical and administrative staff of the Centre. The year under review has been a turbulent time for the Centre with unprecedented departure of a large number of senior staff due to superannuation and deployment elsewhere. However, there is no flagging in the enthusiasm to achieve perfection and commitment to TB control.

I place before you this Annual Report that represents the combined efforts of all the staff members and invite your valuable suggestions to help us improve our research efforts.

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Distinguished Visitors

Date visited	Name	Organistaion/place	Remarks
28.07.08	Dr. D'Aran Richardson	PATH Washington, DC	"Thank you for the very informative visit and the excellent work you are doing! PATH looks forward to continuing our collaboration with you on TB control and elimination!"
28.07.08	Dr. Satish Kaipilyawar	PATH India	"I am impressed as always when I visit TRC, each visit makes me learn new things and motivates me to take on more work. All the best. I am impressed by the system in place at TRC".
19.12.08	Dr. Rajat Goyal	Country Director IAVI	"It's been an exhilarating experience visiting the institute and the personnel. I am convinced about the able leadership influence in the cultural and professional environment of the centre. It's a privilege for IAVI to be a partner organization to an institute of TRC stature".

ABBREVIATIONS

2D-LPE	2D-liquid phase electrophoresis
3TC	Lamivudine
AFB	Acid-fast bacilli
ART	Anti-retroviral treatment
ATP	Adenosine triphosphate
ATT	Anti-tuberculosis treatment
BCG	Bacillus Calmette Guerin
CAS	Central-Asian
CFP	Culture filtrate protein
CMV	Cardamom mosaic virus
CS	Chest symptomatic
CTL	Cytotoxic T-lymphocyte
DC	Dendritic cell
d4T	Stavudine
ddI	Didanosine
DOTS	Directly observed treatment short-course
DR	Drug-resistant
DRM	Drug resistance mutations
DSMB	Data safety and monitoring board
DST	Drug susceptibility testing
EAI	East-African-Indian
EDP	Electronic data processing
EFV	Efavirenz
ELISA	Enzyme linked immunosorbent assay
EMB	Ethambutol
EMSA	Electro mobility shift assay
ESAT-6	Early secreted antigenic target-6
ETH	Ethionamide
FDC	Fixed dose combination
FP	Free probe
FTIR	Fourier transform infrared
GM-CSF	Granulocyte macrophage colony stimulating factor
HHC	Household contact
HLA	Human leucocyte antigen
HO-1	Heme Oxygenase-1
HPLC	High performance liquid chromatography
IGRA	Interferon gamma release assay
INH	Isoniazid
KGDC	KG1 derived dendritic cells
LJ	Lowenstein-Jensen
LRP	Luciferase reporter phage
LTBI	Latent TB infection
MAPK	Mitogen activated protein kinase
mDc	Myeloid dendritic cell

MDR-TB	Multi drug-resistant tuberculosis
MHC	Major histocompatibility complex
MIC	Minimum inhibitory concentration
MLR	Multiple linear regression
MMP	Matrix metalloproteinase
MSM	Men who have sex with men
M. tuberculosis	*Mycobacterium tuberculosis*
NACO	National AIDS control organization
NHS	Normal healthy subject
NK	Natural killer
NRL	National Reference Laboratory
NVP	Nevirapine
ORF	Open reading frame
pDC	Plasmacytoid dendritic cell
PBMC	Peripheral blood mononuclear cell
PCA	Principal component analysis
PLHA	People living with HIV/AIDS
PLS	Partial least square
PMA	Phorbol myristate acetate
PMN	Polymorphonuclear leucocyte
PI	Protease inhibitor
PTB	Pulmonary tuberculosis
RD1	Region of deletion-1
QFT-G	Quantiferon TB gold
RLU	Relative light units
RMP	Rifampicin
RNTCP	Revised National Tuberculosis Control Programme
RT	Reverse transcriptase
RT-PCR	Real-time polymerase chain reaction
SCC	Short course chemotherapy
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error of mean
STPK	Serine/threonine protein kinase
TAM	Thymidine analog mutation
TB	Tuberculosis
TDDRP	Target database for DR pathogen
TIMP	Tissue inhibitor of metalloproteinases
TLR	Toll-like receptor
TRC	Tuberculosis Research Centre
VDR	Vitamin D receptor
WT	Wild type
XDR-TB	Extensively drug-resistant TB
ZN	Ziehl Neelsen

CLINICAL RESEARCH

Ongoing studies

Randomised clinical trial to study the efficacy and tolerability of 3- and 4-month regimens containing moxifloxacin in the treatment of patients with sputum smear and culture positive pulmonary tuberculosis (CTRI/2008/091/000024)

Background

Earlier clinical trials conducted at Tuberculosis Research Centre (TRC) have shown that a 4-month daily regimen which included ofloxacin in the intensive phase was successful in the treatment of sputum positive pulmonary tuberculosis (PTB). But 4-month thrice weekly regimens containing ofloxacin, gatifloxacin or moxifloxacin were less successful with high relapse rates. Following publication of the TRC clinical trial demonstrating the efficacy of an ofloxacin containing regimen in shortening TB treatment to 4 months (Indian Journal of Tuberculosis, 2002), there has been a global interest in the role of quinolones in the primary treatment of TB. Moxifloxacin is now considered as the most effective of the quinolones for the treatment of TB due to its many unique mechanisms involved. TRC is now conducting a randomized clinical trial to study the efficacy and safety of 3- and 4-month moxifloxacin containing regimens for treatment of patients with sputum positive PTB. Newly diagnosed smear positive, PTB patients HIV sero negative for HIV, residing in Chennai and Madurai are randomly allocated to 3-month or 4-month moxifloxacin regimens or a control 6-month regimen. Treatment is given under direct observation and patients are periodically followed up with sputum examinations. The patients are also closely monitored for development of adverse drug reactions. The regimens used in the trial are given in table 1.

Table 1: Trial regimens

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

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

The sample size for the study is 1650 patients, and 270 patients have been enrolled as of 31st March, 2009.

The baseline characteristics of patients recruited to the trial are given in table 2.

Table 2: Baseline characteristics

Regimen	Test regimen 1 (n=52)	Test regimen 2 (n=56)	Test regimen 3 (n=53)	Test regimen 4 (n=54)	Control regimen (n=55)	Total (n=270)
Sex						
Male	40	42	37	39	40	198
Female	12	14	16	15	15	72
Age						
<40 years	33	31	37	35	36	172
≥40 years	19	25	16	19	19	98
Initial sputum smear grade						
0 or 1+	11	14	10	14	9	58
2+ or 3+	41	42	43	40	46	212
No. of zones involved in chest X-ray						
≤ 2	11	13	11	12	12	59
> 2	41	43	42	42	43	211

Preliminary results indicate that a significantly higher proportion of patients treated with the moxifloxacin regimens became sputum culture negative after first and second month of treatment compared to those treated with the control regimen (table 3).

Table 3: Sputum culture results in patients treated with moxifloxacin and control regimens

Month of treatment	Moxifloxacin regimens (n=186)			Control regimen (n=42)		
	No. of patients	Culture negative		No. of patients	Culture negative	
		No.	%		No.	%
1	184	67	36	41	5	12
2	181	171	95	39	28	72

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

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

Background

The TRC has been conducting drug resistance surveillance of patients treated with Revised National Tuberculosis Control Programme (RNTCP) regimens in a TB Unit in Tiruvallur district in Tamil Nadu, south India (1999 to 2005). It was observed that 52 patients failed to Category-II regimen. The drug susceptibility testing (DST) results showed that 32% had multi drug-resistant TB (MDR-TB), 26% had mono or poly drug-resistant (DR) organisms, but were not MDR-TB (i.e. resistant to isoniazid/streptomycin/streptomycin-isoniazid/ethambutol), 29% harboured fully susceptible organisms, and in 13% there was no growth in the culture.

Directly observed treatment short-course (DOTS) using standardized Category-IV regimen (DOTS plus) is being implemented in India in a phased manner. Limited information is available on the feasibility, effectiveness and profile of adverse reactions of the Category-IV regimen. Also, there are no guidelines on the management of patients who fail to Category-II regimen but are non-MDR.

Aims

- To assess the feasibility, effectiveness and profile of adverse reactions of DOTS plus regimen compared to modified DOTS plus regimen in patients who fail to Category-II regimen and have MDR-TB
- To assess the treatment outcome of a re-treatment regimen in patients who fail to Category-II regimen and not having MDR-TB based on drug sensitivity test

Methods

Management of patients with MDR-TB

Eligibility criteria: Patients harbouring *Mycobacterium tuberculosis* (*M. tuberculosis*) resistant to at least isoniazid (INH) and rifampicin (RMP) with or without resistance to other drugs will be eligible for the study. At least one sputum smear examined within 10 days of starting treatment should be positive for acid-fast bacilli (AFB).

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

Regimen I: 6(9) (K_7 (Of, Eth, Z, E & Cy)$_7$ 18 (Of, Eth, E & Cy)$_7$

Regimen II: 6(9) K_3, (Of, Eth, Z, E & Cy)$_7$ 18 (Of, Eth, E & Cy)$_7$

(K – kanamycin; Of - ofloxacin; Z – pyrazinamide; E – ethambutol; Eth – ethionamide; Cy – cycloserine)

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

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

The study patients from Tiruvallur district are hospitalized for the first 2 - 4 weeks of treatment. Treatment is being arranged from the nearest Primary Health

Centre (PHC) to be administered under direct supervision. The drugs are then supplied from TRC to the DOT provider on a monthly basis. All patients from Chennai Corporation are being given treatment as DOT from TRC or its subcentres by TRC staff.

The sample size for the study has been estimated to be about 75 patients in each arm.

Clinical and laboratory investigations

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

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

Outcome measures: The following outcome measures will be analysed:

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

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

Management of patients not having MDR-TB

Background

Eligibility of criteria

Patients, who harbour fully susceptible bacilli, or bacilli resistant to isoniazid/ streptomycin/streptomycin-isoniazid/ethambutol, come under this group.

Treatment regimens

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

Regimen I : 6 $K(REZ)_3$ $(REZ)_7$

Regimen 2: 6 $K(REZH)_3$ $(REZH)_7$

K – kanamycin; H – isoniazid; Z – pyrazinamide; E – ethambutol; R –rifampicin

The sample size for the study has been estimated to be about 75 patients in each arm.

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

Outcome measures: The following outcome measures will be analysed:

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

Treatment failures are being managed based on the DST result. Intake to the study was initiated in September, 2007 and upto 31st March 2009, 58 patients have been recruited (42 in MDR and 16 in non-MDR).

The study is in progress.

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

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

Background

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

Aims

Primary objectives

- To assess the utility of two antibiotic algorithms to improve the efficiency of diagnosis of smear negative TB

- To study the utility of repeat sputum microscopy in chest symptomatics with persistent symptoms after a course of antibiotics

Secondary objectives

- To study the proportion of TB patients among those confirmed by culture, and their correlation with chest X-ray findings
- To obtain information on the etiological profile of respiratory infections and their sensitivity pattern and studying appropriateness of antibiotic algorithm

Methods

Patients referred with cough of >3 weeks and having 3 smears negative for AFB are eligible to get recruited to the study. At TRC, the patients undergo 3 sputum examinations for AFB by smear and culture, and a chest X-ray is taken. They are randomly allocated to one of the following antibiotic regimens for treament duration of 10 days:

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

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

All the patients are being reviewed with culture results. It is proposed to admit 700 patients to each antibiotic arm. Till March 2009, 273 patients have been recruited to the study.

The study is in progress.

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

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

(Funded by Department of Biotechnology, India)

Background

The immunomodulator containing *Mycobacterium w* was developed by the National Institute of Immunology, New Delhi in 1980. It was 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 PTB, showed that the conversion rate was faster when *Mycobacterium w* was added to the short course chemotherapy (SCC). Immunomodulators work against persistors, which may result in reducing the relapse rates. The addition of immunomodulator to chemotherapy is well tolerated and does not increase adverse reactions to the therapy.

Aim

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

Methods

This study was planned as a double blind, randomized, placebo controlled multicentric clinical trial. The patients were randomly chosen to receive either the vaccine or placebo along with the standard Category-II RNTCP regimen.

Results

The study was initiated in March, 2006. In the two year period till March 2008, 268 patients were registered, 115 screened and 59 enrolled into the study. Vaccine acceptability among patients was found to be good. Of the 59 enrolled to the study, 45 have completed treatment and are on follow up, 4 defaulted for treatment - 2 in the intensive phase and 2 in the continuation phase, 4 developed serious adverse events – 2 renal and 2 hepatic. Six patients required change of chemotherapy – 4 for multidrug resistance, one for clinical complication, and one for pregnancy. Two patients who had hepatotoxicity also had change of chemotherapy. Intake to the study has been completed and patients are being followed-up.

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

Preventive therapy for TB among HIV-infected individuals

(Funded by United States Agency for International Development)

Background

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

Aims

- To study the efficacy of two different preventive therapy regimens in HIV-infected persons in reducing the incidence of TB and overall mortality
- To find out if a long duration regimen with INH daily is superior to a 6-month regimen of INH and ethambutol (EMB)

Outcome Measures

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

Study Design

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

The treatment regimens were as follows:

- ♦ EMB (800 mg) and INH (300 mg) daily for six months, self-administered, collected once in fifteen days
- ♦ INH (300 mg) daily for 3 years (in lieu of lifelong prophylaxis) self administered, collected once in fifteen days

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

Patients were followed up for a period of three years from the time of admission to the study. Clinical examination and relevant investigations were done every three months. Patients suspected to have TB at any time were completely evaluated and treated appropriately. Any positive culture was subjected to drug

susceptibility testing. The cause of death was ascertained by a panel of doctors after thorough evaluation of last available records.

Results

Of the 712 patients admitted to the study from March 2001 – September 2005, 683 were eligible for analysis. The mean age, weight, CD4 cell count and Mantoux were comparable in both the groups.

A total of 300 patients from the INH arm and 320 patients from the EMB/INH arm have completed 36 months of follow-up as of 31st March, 2009.

Eighteen patients in the EMB/INH arm developed active TB giving a breakdown rate of 2.11/100 person years and 11 in the INH group (breakdown rate of 1.55/100 person years). Most of the breakdowns in both the arms had occurred within the first 12 months. There were 25 deaths in patients in the EMB/INH arm, and 20 deaths had occurred in patients who were receiving INH (table 4).

The toxicity patterns in both the groups were similar. In only one patient in the INH arm, the treatment had to be terminated because of severe hepatotoxicity culminating in jaundice.

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

Table 4: Incidence of TB and death by regimen (Intent to treat analysis)

	EMB/INH arm	INH arm
TB incidence (per 100 person years)	2.11 (1.42 – 3.46)	1.55 (0.73 – 2.36)
Rate ratio	1.55 (0.79 – 3.03)	1.26 (0.69 – 2.27)
Death (all – cause) (per 100 person years)	2.77 (1.68 – 3.86)	2.21 (1.24 – 3.18)

The above values are mean (range)

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

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

This randomized clinical trial was designed to study the efficacy and safety of two different once-daily anti-retroviral regimens along with ATT in the treatment of HIV-infected TB patients. The specific aim was to compare the efficacy of a once-daily regimen of didanosine (ddI) and lamivudine (3TC) with either efavirenz (EFV) or nevirapine (NVP) when given along with standard ATT in patients with HIV and TB with CD4< 250 cells/mm^3. The primary outcome measure was suppression of viral load to <400 copies/ml at 24 weeks of anti-retroviral treatment (ART). The secondary outcome variable was to compare the utility of directly observed treatment in this setting with self-administered ART.

Recruitment of patients to the trial was initiated in May, 2006 and was stopped in June, 2008 as per recommendation of the Data Safety & Monitoring Board (DSMB). As of April 1, 2008, 564 patients had been screened for the study at 5 centres (3 sites in Chennai, 1 in Vellore and 1 in Madurai). One hundred and sixteen patients were admitted to the study (demographics in table 5). All patients were randomised at the end of intensive phase of ATT (2 months). Fifty nine patients received the EFV-containing regimen and 57 the NVP-containing regimen. Eighty five of these patients had PTB while 31 had extrapulmonary TB (pleural effusion/TB lymph node). Response to ATT was good with 87.5% of patients being culture negative at 2nd month, and 96% of those who completed 6 months of ATT became culture negative. Overall, cure/completion rate was approximately 90%. The favourable response to ATT was 84% in the NVP regimen and 95% in the EFV regimen. Table 6 shows the outcome, by efficacy and intent to treat analysis at 24 weeks of ART. Only one patient had an adverse reaction to ATT drug which required a change of treatment.

The immunological response to ART has been satisfactory with good improvement in CD4 counts (table 7).

The DSMB met on December 15, 2007 (for first interim analysis) and recommended that intake to the NVP arm be withheld till further analysis. This

was in view of the high failure and death of patients admitted to the NVP arm compared to the EFV arm (favourable response 67% in NVP arm vs. 85% in EFV arm, p=0.038).

The second interim analysis was done and presented to the DSMB on June 14, 2008. The DSMB recommended that intake to the study can be stopped, as the primary outcome had been determined to be significantly different between the two regimens.

Table 5: Demographic details

Baseline characteristics*	EFV regimen (n = 59)	NVP regimen (n = 57)
Age (years)	34.5 ± 7.5	37.6 ± 7.8
Weight (kgs)	43.0 ± 8.6	42.0 ± 7.3
Body mass index	16.3 ± 2.6	16.4 ± 2.4
Median CD4 cells/mm^3 (IQR)	85 (47 - 85)	83 (33 - 135)
Median VL copies/ml (IQR)	3,62,000 (41,575-7,50,000)	2,82,000 (1,28,500-6,49,500)

*All values are mean ± SD except CD4 and viral load which are median values and range given in parantheses.

Table 6: Outcome at 24 weeks in the study population

Variable	EFV regimen (n = 59)	NVP regimen (n = 57)
Response to ATT (Efficacy analysis) Favorable	56 (95%)	48 (84%)
Response to ART (ITT analysis) Plasma HIV-RNA < 400 (copies/ml)	50 (85%)	38 (67%)*
Adverse events Any grade Grade 3/4	 65 7	 34 4
Virological failure	6	11
Death	0	5
Lost to follow-up	3	3

* p = 0.038

Table 7: Changes in CD4 counts (cells/mm^3) during ART

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

The above values are mean ± SD

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

Innate and adaptive immunity in children starting antiretroviral drugs in India

(Collaboration with University of Miami, Florida, USA)

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

Background

Currently, CD4 counts are the mainstay of immunologic assessment for HIV-infected adults and children based on which treatment decisions are made. It would be useful to identify other immunologic markers that can be predictive of disease outcome of perinatally HIV-infected children who are treatment naïve but now have access to antiretroviral therapy as per National AIDS Control Organization (NACO) guidelines.

Aims

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

Methods

Between February 15, 2007 and March 30, 2009, 62 ART naïve HIV-positive children (28 males/34 females) were screened and recruited to the study. The

age of the study participants ranged from 9 months to 13 years with a median BMI of 14.4 (range: 6.4 – 24.1). The total CD4 counts in the study subjects ranged from 131 to 2396 (median = 777) cells/mm^3 and CD8 counts from 573 – 6086 (median = 1625) cells/mm^3. During each visit, all children were examined clinically, and venous blood samples were collected for immunological and other parameters as per the protocol.

The number of patients enrolled in the study and their follow-up details as of March, 2009 are given in table 8.

Table 8: Details of patient recruitment and follow-up

	Baseline	1st follow-up (12 weeks)	2nd follow-up (24 weeks)	3rd follow-up (36 weeks)	4th follow-up (48 weeks)
No. of children	62	62	60	54	46

ART was given as per NACO guidelines wherever necessary. Children not requiring ART as per the treatment guidelines were also monitored regularly and provided with prophylaxis/treatment against opportunistic infections. The children are being followed up to 48 weeks, after which they are transferred to the nearest ART centre for further management.

For this analysis, patients were classified into three groups based on their CD4% as immune category-1 (IC-1) [CD4 % > 25], immune category-2 (IC-2) [CD4 % 15 - 25] and immune category-3 (IC-3) [CD4 % < 15]. Eight age matched healthy children (age 3–13 yrs, median = 5 yrs), were also recruited, who served as HIV-negative control subjects. The control subjects underwent a single blood sampling for immunological and other parameters. Statistical analysis was performed using a general linear model with planned contrasts to compare mean values among the three immune categories. SAS version 9.1 was used for all analysis.

Results

HIV-positive children from all the three immune categories (IC-1, IC-2 and IC-3) had a significantly decreased proportion of naïve CD4 and CD8 T-cells compared

to HIV-negative children. In general, as total CD4% decreased, naïve CD4 and CD8 T-cell % also decreased. Proportion of naïve (CD45RA+ CD62L+) cells was greater in CD4 T-cell population as compared to CD8 T-cells, and in both T-cell subsets (CD4 and CD8), the numbers directly correlated with CD4%.

All HIV-positive groups showed a relative increase in expression of CD38 and HLA-DR on CD4 / CD8 T-cells compared to control subjects. CD38 and HLA-DR expression was higher in children belonging to IC-2 and IC-3 compared to those in IC-1. Expression of immune activation markers (CD38+ HLADR+) was greater in CD8 T-cells and its expression remained high during follow-up in both subsets (CD4 and CD8).

Expression of CD127 (IL-7Rα), a marker of memory cells was reduced mainly in CD8 T-cells and its expression was correlated directly with CD4%. Expression of CD127 in CD4+ T-cells was also lower, though not significant.

Myeloid dendritic cells (mDC) from HIV-positive children expressed reduced levels of maturation marker (CD83) upon resiquimod stimulation compared to control subjects. Similar results were obtained with plasmacytoid dendritic cells (pDC) upon stimulation with resiquimod.

No significant differences in cytokine secretion were observed in all the immune category groups. Although induction of cytokines, IFN-α in pDC and TNF-α in both pDC and mDC were normal at baseline, cytokine production was reduced at nine months, predominantly in pDC.

A summary of the key findings of other immunologic markers is presented in table 9.

Conclusions

This cohort of largely clinically stable HIV-infected children has demonstrable immunologic defects dominated by deficits in naïve T-cells and ongoing immune activation, with progressive and subtle defects in innate immunity, involving mainly pDC. All abnormalities showed a gradation in progression from IC-1 group to IC-2 and IC-3, which was parallel to deterioration in immune function (CD4%).

The study is in progress.

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

Table 9: Summary of key immunologic findings

	Baseline compared to controls					At 9months, compared to baseline			
		IC groups					IC groups		
	Total	I	II	III		Total	I	II	III
DC	↔	↔	↔	↔		↔	↔	↔	↔
pDC CD80	↔	↑	↔	↔		↔	↓	↔	↔
mDC CD83	↓	↓	↓	↓		↔	↔	↓	↔
pDC CD83	↓	↓	↓	↓		↔	↔	↔	↔
pDC IFNα	↔	↔	↔	↔		↓	↓	↓	↔
mDC TNFα	↔	↔	↔	↔		↓	↔	↔	↔
pDC TNFα	↔	↔	↔	↔		↓	↓	↓	↓
CD4 DR+ CD38+	↑	↔	↑	↑		↔	↑	↔	↔
CD8 DR+ CD38+	↑	↔	↑	↑		↔	↑	↔	↔
CD4127	↔	↔	↔	↔		↑	↑	↔	↔
CD8127	↓	↔	↔	↔		↑	↑	↔	↔
CD8 Naïve	↓	↓	↓	↓		↔	↔	↔	↑
CD4 Naïve	↓	↓	↓	↓		↔	↔	↔	↔
CD4 Central Memory	↔	↔	↔	↔		↓	↔	↔	↔
CD8 Central Memory	↔	↔	↔	↔		↔	↓	↓	↔
CD4 Effector Memory	↔	↔	↔	↑		↔	↑	↔	↔
CD8 Effector Memory	↑	↔	↑	↑		↓	↔	↔	↔
CD4 Effector	↔	↔	↔	↔		↔	↔	↔	↔
CD8 Effector	↔	↔	↔	↔		↔	↔	↔	↓

↔ no difference, ↑ increase, ↓ decrease

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

(Collaboration with YRG Care, Chennai and NIAID, NIH, USA)

[Funded by National Institute of Allergy and Infectious Diseases (NIAID)]

The goal of this study is to determine the changes in HIV viral load that occur in patients co-infected with HIV and filaria, over 1 year, following treatment with DEC/Albendazole, and to compare those with changes in viral load among HIV-infected patients without filarial co-infection. Two groups of patients are being recruited for the study; the first is a group with HIV and filarial infection (detected by serum antigen test) and the second, or control group have HIV infection, but not filariasis. The second group of patients is matched with the first group based on age, gender, HIV viral load and CD4 cell counts. The total sample size required for this study is 138 (HIV/Filarial – 46, HIV – 92). Patient recruitment is being done at both TRC and YRG Care. Screening of patients for this study started on May 15, 2007 at TRC and up to March 31 2009, 254 patients were screened, of whom 32 (HIV and Filaria – 10, HIV - 22) patients have been recruited to the study. Patient enrolment to the study is ongoing.

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

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

(Collaboration with Government Hospital for Thoracic Medicine, Tambaram and National AIDS Research Institute, Pune)

[Funded by United States Agency for International Development (USAID) through the WHO under the Model DOTS Project]

Background

In HIV-infected patients with active TB disease, sputum smears are more likely to be negative for AFB by smear microscopy. In RNTCP, diagnosis of TB is based on sputum smear examination and response to a course of antibiotics.

Aim

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

Methods

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

Results

Out of 270 patients recruited in to the study, 249 (males – 167; females – 82) were included in the analysis. The mean age and mean body weight of the patients were 35.8 years and 46.7kg respectively. The median CD4 cell counts were 182 cells/mm^3. About 77% of the patients had cough and breathlessness for > 3 weeks, 64% had fever for > 3 weeks, and 89% had weight loss. Forty eight patients (19.3%) had a positive TB culture initially. Of the 144 patients (58.5%) with an abnormal chest X-ray, 45 (36.6%) demonstrated clinical and radiographic improvement after treatment with antibiotics.

Patient recruitment at Chennai has been completed, while it is ongoing at the National AIDS Research Institute, Pune.

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SOCIOLOGICAL RESEARCH

Completed Studies

Perceptions of patients on routine referral of TB patients for HIV testing - A study from south India

(Funded by Central TB Division, New Delhi)

Summary

TB patients living in HIV high prevalence areas in India have been offered HIV testing since early 2008. This study described the findings of a pilot project, focusing on understanding patients' perspectives on the process of referral for HIV testing and the problems encountered. This was a cross-sectional study conducted in 2 districts. Government health staff were trained to assess the HIV status of all TB cases and refer all persons with unknown HIV status to the nearest HIV counseling and testing centre free of cost.

Of the 568 patients, who were interviewed, 455 (80%) reported being referred for HIV testing after they presented to the TB clinic for investigations or for treatment of TB. Of the 110 HIV-infected patients interviewed, 89 (81%) were referred to the ART centre and 82 (92%) actually went to the ART centre, despite the distance to the testing centres and financial difficulties. Patients expressed problems with regard to frequent visits.

This study provided the first evidence from India that routine, provider-initiated voluntary HIV testing of TB patients can be achieved with very high efficiency under programmatic conditions.

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

A situational analysis of health seeking behavior and awareness of tuberculosis among migrants – Brick kiln workers - A study from Tiruvallur district, Tamil Nadu, India

[Funded by United States Agency for International Development (USAID) through the WHO under the Model DOTS Project]

Migration has been an important phenomenon in managing public health issues as migrants find it difficult to access various health care programmes. This

phenomenon has been a challenge to the TB control programme also. During the survey conducted by TRC in the Model DOTS area, it was evident that there were many temporary migrants in this area, which included brick klin chamber workers, seasonal farm workers, construction workers and lay workers. Among them, majority however were brick kiln workers. Since the brick kiln chambers are situated on the outskirts of the main villages and towns, accessibility to health care becomes extremely difficult for the brick kiln workers.

There is a dearth of information on the prevalence of TB among the brick kiln workers, their awareness of the disease, and the health seeking behaviour of chest symptomatics. A qualitative situational analysis of this group was undertaken in Tiruvallur district of Tamil Nadu during March-June 2008 to understand the profile of brick kiln workers, such as their background, access to health care, general health seeking behaviour especially related to TB. One hundred and seventy brick kilns were randomly selected.

The study findings highlighted some of the challenges faced by the brick kiln workers with regard to their health, health seeking behaviour and access to health care services. The nature of their occupation, with exposure to heat and dust for long hours, makes them particularly vulnerable to respiratory illnesses. The public sector health facilities were open during the day when the brick kiln workers were at work in the chambers which made it difficult for them to access health care. To solve these problems, the brick kiln owners were linked with the local private health facilities. This limited the possibility of the workers receiving proper health management. The public sector health providers expressed difficulties in providing care to this group of migrant workers, especially if they presented with symptoms related to TB, that demanded further investigation. The availability of smear microscopy services only at the main PHC, which is usually at a considerable distance from the chambers, led to long delays in investigations, diagnosis, and patients not reporting back to collect their results and intiating TB treatment, if required. The provision of DOTS services once again became a challenge due to the temporary nature of the workers' jobs in one place.

This study has provided an initial insight into the challenges faced by brick kiln workers in terms of access to care. It also brings out the constraints faced by health providers in TB management among this group. However, this is a preliminary report and provides the background for a more detailed quantitative study on the number of chest sympotmatics among brick kiln workers, health seeking behaviour among chest symptomatics, awareness of TB among workers and the owners, prevalence of TB among brick kiln workers and TB management.

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

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

(Collaboration with UCLA - University of Los Angeles, USA)

Summary

The purpose of this study was to explore the perceptions and needs of mothers living with HIV to gain greater insights into the challenges they face in relation to their health seeking behavior, fears around disclosure, and issues related to stigma and discrimination. This qualitative study utilized focus groups consisting of a sample of 60 HIV-infected mothers recruited from a large maternity hospital and State TB Demonstration clinic in Chennai, India. The study particpants expressed discrimination by physicians and other health care workers a major impediment in accessing quality health care. Mothers living with HIV were increasingly concerned about how and when to disclose their HIV status to their children, and the repercussions which could result upon disclosure. The findings of this study call for intervention strategies, taking into consideration the various concerns and needs of mothers living with HIV and their children.

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

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

[Funded by United States Agency for International Development (USAID) through the WHO under the Model DOTS Project]

Summary

The TB control programme is based on passive case finding. It is therefore crucial to understand the health care seeking behaviour of chest symptomatics (CSs). In 1997, prior to the implementation of the RNTCP, a community based study of the care seeking behaviour of the CSs from urban and rural areas in Tamil Nadu was carried out. This care seeking behaviour of urban and rural populations following implementation of RNTCP was examined in the present study using the same methodology. The aim of the study was to compare the care seeking behaviour of CSs, type of health providers they approached, the factors that influence the choice of providers before and after the implementation of the RNTCP. In addition, information was collected on the knowledge of TB.

The prevalence of CSs in urban and rural areas was 2.7% and 4.9% respectively. Prevalence was found to increase with age. The percentage of CSs living in urban and rural areas, reporting to the private care facilities as their first point of contact were 58.7% and 41.3% respectively, and of those who utilized Government facility was 36% and 64% respectively; these differences were highly significant. The salient findings of this study were that, after implementation of the RNTCP, the number of CSs reporting to the private care facilities initially has been reduced and persons seeking care in the Government facility has significantly increased. It was also encouraging to note that 69% of the rural respondents as compared to 61% of urban respondents were aware that treatment for TB was available close to their residence. However, the fact that among those who approached Government facilities, only a quarter of respondents opted for repeat visits to the government clinics and shifted to private care or other alternative systems of medicine is a matter of concern. Dissatisfaction with health providers was the main reason for the shift in care provider. The findings call for strengthening the revised control program keeping in mind these issues.

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

Pediatric disclosure: Family caregiver's perceptions on disclosure of HIV infection to children living with HIV

Summary

With the introduction of ART and frequent visits to the ART clinics, many caregivers are facing the challenge of whether and how to disclose the HIV status to their children living with HIV. A cross-sectional study was undertaken in which 65 caregivers of 95 HIV-infected children registered in two pediatric clinical studies at TRC from 2000 to 2007 participated. About 88% of the caregivers had not disclosed to the child that he/she was infected with HIV. Nearly three quarters of the respondents said that they would disclose when the child was older, and were of the opinion that the best age for disclosure of HIV diagnosis to children was between 12 and 15 years. A majority of care givers requested help with regard to disclosure. The findings call for intervention strategies in care givers with regard to disclosure of HIV status to their children.

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

Ongoing studies

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

(Collaboration with Harvard Medical School/MGH and Fenway Community Health)

(Funded by Indo-US Joint Working group)

Men who have sex with men (MSM) in India are a marginalized population who are in need of evidence based HIV prevention efforts. MSMs are considered "bridge" populations, where wives of MSMs, clients of sex workers, patients with sexually transmitted diseases and partners of drug users, over a time period, "bridge" the virus from highest risk groups to the general population. Our initial study on the behavioural risk factors among 210 MSMs provided a background that is relevant to the conduct of the present study. The innovativeness of this study is that it explores the possibility of providing an intervention which targets

psychosocial problems concurrent with HIV risk reduction behaviours among the MSM population.

Aims

- To develop a behavioural prevention intervention for MSMs in Chennai
- To conduct a randomized controlled study on the intervention in MSMs in Chennai with an outcome of risky sexual behaviour

This project will help to develop a model for an effective holistic intervention for MSMs, which will be contextual and culture specific. In addressing these concerns it is expected that attempts can be made towards reduction in HIV transmission.

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

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

With the introduction of ART and the need for life long treatment, HIV-infected parents are faced with the biggest challenge on how to disclose their (parents) HIV status to their children.

There is some evidence that HIV disclosure, though stressful, facilitates emotional support which may lead to more effective coping and enhanced psychological adaptation. However the fear of stigma and discrimination faced by the children of PLHAs (people living with HIV/AIDS) may inhibit parents from disclosing their HIV status to their children and others.

It is against this background that this cross sectional study was carried out covering PLHAs attending the out patient TRC clinics after obtaining their consent. In-depth interviews were conducted using a semi-structured interview schedule. One hundred and ten patients were recruited and interviewed. The analysis of the study is in progress.

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

A study on sexual behaviour among sero-discordant individuals

There is dearth of information in India on sexual behavior and sexual risk factors among HIV sero-discordant couples. Hence a study was undertaken to understand the sexual behaviour patterns and sexual risk factors for HIV transmission which would be useful for health care providers in dealing with this group of individuals. This qualitative study aimed to explore sexual behaviour patterns among sero-discordant individuals. In-depth interviews were conducted among eligible patients after obtaining their consent. Patients were recruited from outpatient clinics of TRC and TRC subcentres at Government hospitals in Chennai and Vellore. Eighty five interviews have been conducted and analysis of the study data is in progress.

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

PHASE 1 PREVENTIVE HIV VACCINE TRIAL

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

The results of the first phase I HIV vaccine trial using a multigenic MVA vaccine conducted at this centre indicated that while this was safe and effective in inducing immune response in all the vaccine recipients, the persistence and the magnitude of the response should be improved before moving this vaccine further in human trials. Therefore, it was decided to use this in a prime-boost regimen involving a DNA vaccine (ADVAX) to prime and the MVA vaccine to boost. During the year under review, the necessary regulatory clearances were obtained and the laboratory procedures were standardized. The advocacy activities were initiated in March, 2009.

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

EPIDEMIOLOGICAL & OPERATIONAL RESEARCH

Completed studies

Spoligotyping *M.tuberculosis* isolates from Tiruvallur district

[Funded by United States Agency for International Development (USAID) through the WHO under the Model DOTS Project]

Background

The last decade has seen a dramatic resurgence in the incidence of TB throughout the world and an increased need for more rapid methods to diagnose and prevent dissemination of this disease. Spoligotyping, a method for simultaneous detection and typing *of M. tuberculosis* complex bacteria, has been recently developed. The clinical usefulness of spoligotyping is determined by its rapidity, both in detecting causative bacteria and in providing epidemiologic information on strain identities.

The SpolDb4 defines 22 lineages and sub lineages. The previously defined Central-Asian (CAS) lineage has been split into CAS1-Delhi type (ST26) found mainly in the Indian subcontinent and CAS1-Kilimanjaro (ST21) found in Tanzania. Within the East-African-Indian (EAI) lineage, new prototypic spoligotyping-signatures for four sub lineages are presented (EAI2-Nonthaburi, EAI6-Bangladesh/1, EAI7-Bangladesh/2 and EAI8-Madagascar). The EAI2 clade has been designated as the "Manila family". EAI3 and EAI4 are now being shown as phylogeographically specific from India and Vietnam respectively, with suggested designations of EAI3-IND and EAI4-VNM.

Two new lineages from Bangladesh are found, designated as EAI6-Bangladesh/1 (58.1% of isolates from Bangladesh) and EAI7-Bangladesh/2 (91.2% of isolates from Bangladesh). EAI6-BGD1 harbours specificity for the eastern part of the South Asian region since it is also found in neighbouring Myanmar (results not shown).

Aim

- To analyse our spoligotyping data with the newly available classification

Results

Out of the 2000 samples, 1757 isolates were available for spoligotyping. Tables 10A & 10B gives the details of spoligotype distribution in Tiruvallur district that was analyzed using SpolDb3 and SpolDb4 data bases respectively.

The orphan strains correspond to 30.6% and EAI3_IND correspond to 28.9%, followed by EAI5 19 %. EAI6_BGD1 and EAI1_SOM were 5.2 and 4% respectively.

Conclusions

The initial 1362 samples analyzed by SpolDb3 database using Spotclust software showed an equal predominance of EAI 3 and EAI 5 spoligotypes. But our recent analysis using the spolDb4 database has shown that EAI3_IND is 28.9% (reduced by 10%) due to single spot variations. The earlier percentage of EAI 5 (19%) was changed to 21% due to reclassification into EAI1_SOM, EAI6_BGD1, EAI5 or EAI3, EAI2_MANILLA, MANU1 and EAI undefined.

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

Table 10: Analysis of spoligotyping using SpoIDb3 and SpolDb4 data bases

10A: Analysed according to SpoIDb3

Spoligotype	Number of isolates	%
LAM7	1	0
M.microti	1	0
X2	1	0
X3	1	0
EAI1	2	0.17
Family36	2	0.17
H37RV	2	0.17
Orphan	2	0.17
T2	2	0.17
EAI 2	4	0.33
EAI5	4	0.33
EAI3	6	0.5
LAM9	6	0.5
EAI4	7	0.57
Orphan	7	0.57
LAM8	8	0.6
EAI2	14	1.2
Haarlem1	14	1.2
Family 33	17	1.4
Orphan	20	1.6
Beijing	24	2
T1	36	3
CAS	47	3.9
EAI 3	488	40.3
EAI 5	499	41.2
Total	**1212**	

10B: Analysed according to SpoIDb4

Spoligotype	Number of isolates	%
H37Rv	1	0
LAM1	1	0
LAM9	1	0
T1-T2	1	0
Orphan	1	0
EAI2_NTB	2	0.11
EAI8_MDG	2	0.11
LAM5	2	0.11
T3	2	0.11
T4	2	0.11
T5	2	0.11
X2	2	0.11
MANU1	3	0.17
S	3	0.17
EAI undefined	4	0.23
H1	4	0.23
H3	4	0.23
CAS	5	0.3
EA12_MANILA	6	0.35
CAS2	7	0.4
U	18	1
EA15 or EA13	24	1.4
T1	32	1.8
BEIJING-LIKE	36	2
CAS1_DELHI	51	2.9
EAI1_SOM	70	4
EAI6_BGD1	91	5.2
EAI5	342	19
EAI3_IND	508	28.9
Orphan	534	30.6
Total	**1757**	

Ongoing studies

Epidemiological impact study: disease survey

[Funded by United States Agency for International Development (USAID) through the WHO under the Model DOTS Project]

Background

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

Aim

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

Methods

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

Results

Three serial disease prevalence surveys have already been completed. The fourth survey was started in June, 2006 and is in progress. Coverage in this survey was above 90% for all examinations namely symptoms, chest X-ray and sputum examination. The coverage upto the period March, 2009 is shown in table 11.

Table 11: Coverage for examinations – fourth survey (till March, 2009)

Activities	Number of individuals
Enumeration	1,07,521
Symptom screening	95,619
X-ray screening	95,270
Sputum eligible	11,270
Sputum collection	10,857

Three hundred and seventy four individuals were identified as cases through sputum examination either by smear, culture or both.

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

One time prevalence survey

Background

The reduction in prevalence of TB over a period of 10 years in the Tiruvallur area is contributed by the impact of DOTS strategy and continuous active surveillance for TB in this area. In order to measure the impact due to DOTS strategy alone, a one time survey has been planned in the villages that are not covered by the impact survey, but which have been included in the original Bacillus Calmette Guerin (BCG) trial area.

Aim

- To measure the contributions of the prevalence survey itself and other confounders to the documented decline in prevalence

The sample size for this survey was estimated to be 54110 (entire population). The survey methodologies are the same as that of the epidemiological impact survey. So far, 19251 persons have been covered. The survey is in progress.

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

Training activities

The TRC has set up a training and demonstration centre at Tiruvallur which is being used for training health workers deputed from various institutions in conducting disease and tuberculin surveys. The duration of training for disease survey lasts for one month, while it is two months for tuberculin survey. In the last two years, the epidemiology division at TRC has trained health workers in the conduct of the disease survey from the following institutes:

1. NTI, Bangalore
2. RMRCT, Jabalpur
3. MGIMS, Wardha
4. PGIMS, Chandigarh
5. AIIMS, New Delhi
6. JALMA, Agra

Likewise, health workers deputed by the following institutes were trained in conducting tuberculin survey:

1. LRS Institute, New Delhi
2. NDTB Centre, New Delhi
3. CMC, Vellore
4. MGIMS, Wardha

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

APPLIED RESEARCH

Completed studies

Determination of the binding ability of activated INH with AccD6 from *M. tuberculosis*

Experiments based on DNA micro array have provided information on the involvement of *accD6* (an important component of mycolic acid synthesis) with activation of INH. In order to understand the role of *accD6* gene, structural prediction of AccD6 protein and its docking with activated INH were undertaken. Homology modeling of AccD6 was performed using the software-MODELLER9v3, and docking of acetyl-CoA (substrate) and activated INH (inhibitor) was carried out by two softwares-GOLD 4.0.1 and AUTODOCK 4.0.5. The chosen template (2A7S) had a sequence identity of 43% for modeling AccD6 from *M. tuberculosis*. The generated model was subjected to validation by Ramachandran Plot using RAMPAGE and combinatorial extension method. The docking of acetyl-CoA with AccD6 (-63.82kcal/mol) was possible with GOLD, whereas the docking of isonicotinic acid was not feasible due to spatial inconsistency. The successful docking of both the substrate and inhibitor with AccD6 was performed with AUTODOCK displaying (acetyl-CoA) + 1536.92kcal/mol and (INADH not isonicotinic acid) -7.23kcal/mol as scores. Therefore, we have presented the model structure of AccD6 and made an attempt to predict a primary binding site for activated INH (INADH) in AccD6. The results suggest that AccD6 could also be the target for activated INH in addition to InhA and KasA.

[Contact person: Dr.N. Selvakumar (E-Mail ID: selvakumarn@trcchennai.in)]

Field evaluation of concentrated method for improvement of ZN staining techniques

Background

For over a century, the diagnosis of PTB is being confirmed by detection of AFB in direct smears of sputum made on glass slides. Good laboratory practices have to be followed while making direct smears from the mucopurulent portion of sputum on glass slides in order to avoid laboratory acquired TB infection. There is a felt need to develop a new technique to stain the AFB, which would be both simple and non-hazardous. Recently, it has been shown that the deposit of sputum sample, obtained after decontamination with 4% sodium hydroxide and concentrated by centrifugation, can be stained with 1% carbol-fuchsin in its container. The smear can be made subsequently on a glass slide, decolourised and counter-stained by the procedure followed in the Ziehl Neelsen (ZN) method.

Aim

- To stain the sputum sample in its container with phenol ammonium sulphate carbol-fuchsin with subsequent decolourisation and counter-staining of its smear on glass slide for detection of AFB

Methods

A total of 560 sputum samples collected from patients attending a TB clinic were selected. Direct smears were made and stained by the conventional ZN staining method. The samples were then treated with 1-2 ml of phenol ammonium sulphate basic fuchsin solution and left at room temperature for 90 minutes. Later, smears (sediment smear) were made, air dried, and decolourized and counterstained using 25% sulphuruic acid and 0.1% methylene blue. The sediment and direct smears were coded and read, and the results were compared.

Results

The yield of AFB positive smears was similar in direct and sediment smears and the difference was not significant (table 12).

Table 12: Comparison of sediment smears with direct smears

		Sediment smears*					
Direct smears		Negative	1+	2+	3+	Scanty	Total
	Negative	473	0	1	6	0	480
	1+	7	12	9	6	3	37
	2+	2	6	5	4	1	18
	3+	2	5	1	12	3	23
	Scanty	0	0	0	2	0	2
Total		484	23	16	30	7	560

* Negative = no AFB in 100 fields; scanty = 1- 9 AFB in 100 fields; 1+ = 10 – 99. AFB in 100 fields; 2+ = 1 to 9 AFB per field in at least 50 fields; 3+ = more than 10 AFB per field in at least 20 fields.

[Contact person: Dr.N. Selvakumar (E-Mail ID: selvakumarn@trcchennai.in)]

Profile of sputum samples submitted for acid-fast bacilli microscopy in a semi-urban TB clinic, Tamil Nadu, India

Background

The profile of sputum samples submitted by PTB suspects in a TB clinic during a 1 year period, and its influence on smear results was studied.

Aim

- To study the 'type', 'quantity' and 'quality' of sputum specimens submitted by the PTB suspects attending a TB clinic and their influence on AFB smear results

Methods

Sputum specimens were collected from patients attending the TB clinic at Poonamallee. During the 12 month period, from November 2007 to October 2008, laboratory technicians trained at the National Reference Laboratory (NRL) recorded the 'quality', 'quantity' and 'type' of sputum submitted by patients. The laboratory technicians examined and reported the results of sputum smears stained by the hot ZN method. Sputum samples from all smear positive patients were analysed to know the influence of sputum profile on AFB smear results.

Results

Distribution of smear results of 579 samples from 221 sputum smear-positive patients revealed that 352 (60.8%) samples were saliva, 364 (62.9%) contained

more than 2 but less than 4ml of sputum. The yield of AFB positives was, similar for mucoid (200/219 - 91.3%) and saliva (326/352 - 92.6%), and higher in samples with more than 4ml quantity (178/180 – 99.4%), and in morning samples (178/180 – 99.4%).

Conclusion

The yield of AFB positive smears in sputum smear positive patients was similar in mucoid and salivary samples, high in samples containing >4ml volume, and in samples collected in the morning. The 'type' and 'quantity' rather than the 'quality' of sputum play a significant role in detection of AFB positive smears.

[Contact person: Dr.N. Selvakumar (E-Mail ID: selvakumarn@trcchennai.in)]

Age, nutritional status and CYP2B6 G516T polymorphism influence nevirapine blood levels in HIV-infected children on generic anti-retroviral treatment

(Collaboration with BJ Wadia Hospital for Children, Mumbai, Govt. Hospital of Thoracic Medicine, Chennai and Govt. Rajaji Hospital, Madurai)

Background

Most ART programs in resource limited countries use NVP based generic fixed dose combinations (FDC) for treatment, because of affordability, ease of administration and lack of teratogenecity. In India, antiretroviral drugs for pediatric use were made available by the NACO at the Government ART centres only from November, 2006; prior to that, adult formulations were used. These specially formulated pediatric generic drugs are available as FDC, consisting of stavudine (d4T) with 3TC and NVP formulated in two different ratios (6:30:50 & 10:40:70 mg respectively). It is however, important to ensure that children receive adequate doses, and plasma concentrations of antiretroviral drugs are maintained within the therapeutic range.

Some of the factors known to influence NVP drug levels include age, co-administration of other drugs and pharmacogenetic variability. It has been suggested that in general, adult FDCs, while suitable for older children, are not suitable for very young children. Not many studies have specifically examined

the bio-availability of pediatric FDCs and none have been conducted in Indian children. Further, the relationship of drug levels with moderate and severe grades of underweight and stunting has not been well studied.

Aim

- To examine the influence of age, sex, drug dose, nutritional status and CYP2B6 G516T polymorphism on blood levels of NVP in HIV-infected children treated with generic antiretroviral drugs

Methods

This was a multi-centric study conducted at four sites in India. Ninety four HIV-infected children, aged 6 months to 14 years, receiving generic NVP-based FDCs from the out-patient clinics at the Government Rajaji Hospital, Madurai, B.J.Wadia Hospital, Mumbai, Government Hospital of Thoracic Medicine, Tambaram and Kilpauk Medical College and Hospital, Chennai were recruited. Trough and 2-hr NVP plasma concentrations were determined by high performance liquid chromatography (HPLC) and genotyping of CYP2B6 G516T polymorphism by direct sequencing.

Results

The trough and two-hr NVP concentrations in the different groups of children are given in table 13. Stunted children had significantly lower 2-hr NVP concentration compared to non-stunted ($p<0.05$). NVP levels were significantly higher in TT compared to GG and GT CYP2B6 genotypes ($p < 0.01$). Children below three years had a 3.2 times (95% CI: 1.07 – 9.45) higher odds of having sub-therapeutic NVP levels.

Conclusions

A combination of factors such as young age, CYP2B6 GG/GT genotype and stunting could result in sub-therapeutic NVP levels in children. A substantial proportion of children had levels below the generally accepted lower therapeutic limit of 3.0µg/ml and this was more pronounced in the younger children. This is a matter of concern and is likely to play a role in long-term viral control. The study findings suggest that higher dose recommendations may be required for malnourished (stunted) children and those below three years of age.

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

Table 13: Plasma NVP concentrations [median, (Q1, Q3)] in the different groups of children

Groups	N	Trough concentration (µg/ml)	N	2-hr concentration (µg/ml)
Sex				
Female	41	3.57 (2.27, 5.24)	39	4.98 (3.10, 8.12)
Male	47	3.71 (2.39, 5.24)	48	5.96 (4.17, 7.89)
Dose				
<300 mg/sq.m/day	40	3.32 (2.34, 4.66)	40	5.29 (3.03, 6.21)
≥300 mg/sq.m/day	48	4.10 (2.34, 6.09)	47	6.16 (4.30, 9.50)
Drug formulations				
d4T : 3TC : NVP 6 : 30 : 50 10 : 40 : 70 30 : 150 : 200	 24 25 39	 3.47 (2.12, 4.52) 3.33 (2.32, 4.53) 4.50 (2.58, 6.12)	 22 27 38	 5.19 (3.40, 6.49) 5.33 (3.00, 8.59) 6.06 (4.06, 10.09)
Height-for-age Z score				
Stunted (< - 2 HAZ)	55	3.55 (2.39, 4.81)	55	5.29 (3.24, 6.87)*
Non – stunted	33	3.91 (2.32, 6.43)	32	6.08 (4.98, 10.90)
Weight-for-age Z score				
Underweight (< - 2 WAZ)	51	3.71 (2.42, 5.24)	53	5.61 (3.88, 7.77)
Normal weight	37	3.20 (2.27, 5.28)	34	5.63 (3.26, 8.09)
Age				
≤ 3 years	17	2.52 (1.75, 3.80)*	14	4.21 (2.59, 5.81)*
3 years	71	3.98 (2.47, 5.53)	73	5.74 (3.99, 8.35)

* denotes $p<0.05$

Phages and phage lysin to control overgrowth of normal flora in processed sputum specimens grown in liquid medium

Background

Rapid diagnosis of TB is essential to control the spread of TB especially in MDR-TB patients and in those co-infected with TB and HIV. The overgrowth of normal flora escaping the action of sputum processing chemical such as 4% sodium hydroxide is a major problem in rapid broth-based detection systems, affecting the sensitivity of any rapid assay. Use of phagebiotics to overcome this problem has been established and it forms a novel, bio-friendly approach to tackle non-mycobacterial contaminants.

Aim

- To strengthen phagebiotics by use of phage lysin for complete and effective control of normal flora in sputum specimens and to evaluate the same in retrieval of *M. tuberculosis* by luciferase reporter phage (LRP) assay

Methods

Crude lysin was prepared from phage host mixture using standard procedures. One hundred and twenty sputum samples processed using 4% sodium hydroxide were collected and divided in to four aliquots after inoculating on to blood agar plates (Stage I). Nutrient broth was added to one part (Stage II) that served as control. To the other three parts, phagebiotics (Stage III), phagebiotics-lysostaphin (Stage IV) and phagebiotics-lysin (Stage V) were added, randomized, incubated at 37°C for 18-24 hrs and inoculated on blood agar plates. The effect of lysin on retrieval of *M. tuberculosis* up to 3 days by LRP assay was tested using the standard protocol.

Results

The phagebiotics supplemented with lysin arrested the growth of surviving normal flora in more number of samples (112) when compared to Stage II (12), Stage III (70) and Stage IV (81); the difference was statically significant (table

14). Lysin did not show any inhibitory activity on *M. tuberculosis* $H_{37}Rv$ and clinical strains of *M. tuberculosis*.

Conclusion: Phagebiotics supplemented with lysin can be used to control the overgrowth of normal flora in liquid media without compromising on the viability of *M. tuberculosis*.

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

Table 14: Growth of normal flora surviving after processing of sputum samples

Growth on blood agar	Number of samples				
	Stage I	Stage II	Stage III	Stage IV	Stage V
Confluent growth, mixed	0	105	5	3	0
Bacillus sp	0	0	24	23	3
Staphylococcus sp	26	3	21	13	5
No growth	94	12	70	81	112
Total number of samples	120				

Stage I: Soon after processing of sputum samples

Stage II: Grown overnight in nutrient broth

Stage III: Grown overnight in phagebiotics

Stage IV: Grown overnight in phagebiotics supplemented with lysostaphin

Stage V: Grown overnight in phagebiotics supplemented with lysin

Isolation of active compounds from essential oils against *M. tuberculosis* using bioassay guided fractionation

(Funded by Council of Scientific and Industrial Research, New Delhi)

Background

Search for novel and more effective anti-TB agents is important in order to tackle MDR strains of *M. tuberculosis*. Plants are of diverse chemical nature and form a valuable source of new antimycobacterial drugs or a lead compound from which new drugs may be developed.

Aims

- To isolate the active fraction against *M. tuberculosis* from cinnamon oil using bioassay guided fractionation method
- To determine the minimum inhibitory concentration (MIC) of the isolated active compound against clinical isolates of *M. tuberculosis*

Methods

Based on antimycobacterial screening by LRP assay, cinnamon oil was chosen to be the most promising oil, and was subjected to further fractionation and phytochemical analysis. The oil was fractionated by high vacuum distillation and purified using different chromatographic techniques. Based on bioassay guided fractionation, one active molecule was identified, and spectral analysis was performed to elucidate the structure. The MIC was determined for the active molecule against drug sensitive and drug-resistant clinical isolates of *M. tuberculosis.*

Results

Four fractions were obtained from cinnamon oil of which fraction III showed high activity (96.11%) against *M. tuberculosis* even at a low concentration of 50 μg/ml (table 15). Based on spectral analysis, the active fraction was identified as *trans*-cinnamaldehyde. The MIC value of *trans*-cinnamaldehyde ranged from 5-100μg/ml for drug sensitive and drug-resistant clinical isolates of *M. tuberculosis*.

Conclusion

The results suggest that cinnamaldehyde has good antimycobacterial activity. It is worth pursuing further studies using this compound to evaluate its potential as a potent anti-TB agent.

Table 15: Percentage reduction in relative light units (RLU) by different fractions of cinnamon oil against *M. tuberculosis*

Fractions	Concentration	
	50 μg/ml	100 μg/ml
I	31.15 ± 1.56 [d]	42.37 ± 3.29 [c]
II	55.41 ± 2.77 [b]	58.30 ± 0.33 [b]
III	96.11 ± 0.36 [a]	98.78 ± 0.03 [a]
IV	48.96 ± 1.14 [c]	61.38 ± 1.17 [b]

Values represent mean % reduction in RLU ±SD of two replicates

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

Lytic efficiency of mycobacteriophages

Background

The emergence of resistance to mycobacteria by currently available antimicrobial agents prompted interest in searching for alternatives to conventional drugs. One possible option is to use bacteriophages. They are often highly specific and are nontoxic to animals and plants. An attempt was made to determine their efficiency in killing the host bacteria. The performance of five phages namely, D29, TM4, I3, Che7, Che11 was tested in killing clinical strains of *M. tuberculosis*.

Aims

- To determine the efficiency of five lytic mycobacteriophages in killing clinical strains of *M. tuberculosis*
- To evaluate the lytic efficiency of D29 and TM4 based on codon usage analysis

Materials and methods

Suspensions were made from 10 clinical strains of *M. tuberculosis* in Middlebrook 7H9 liquid medium supplemented with bovine serum albumin and divided into 6 aliquots of 1.5 ml each. 300 μl of high titre phages namely, D29, TM4, I3, Che7, Che11 was added to each of the aliquots. A negative control was included. After 3, 6, 24 and 48 hrs of incubation, 250 μl of the mixture was removed and added to 50 μl of phAE129. RLUs were measured using a cuvette luminometer after three hrs of incubation. Codon usage of TM4 and D29 was analyzed using CodonW. Blast and Pfam were used for database search and domain analysis. ClustalW was used for multiple sequence alignment was used.

Results and conclusion

D29 phage was found to be most effective in killing all the 10 clinical strains tested. The Chennai phage Che7 was able to kill 8 of the 10 mycobacterial strains while TM4 was least effective.

Based on codon usage analysis, the genome of TM4 was identified to have high codon usage bias, revealing that the genes of TM4 have better translational efficiency than D29. This better translational efficiency is expected to quicken the lysis process also probably qualifying it to be a better lytic phage. However, the lytic performance of TM4 was poor in all the strains tested in the present study.

In order to understand the reasons for the poor performance of TM4 contradictory to the prediction by the whole genome analysis, sequence analysis of holin and lysin genes was done. It was found that the observed low lytic activity of TM4 may be due to the following reasons:

- The presence of highly charged N-terminal region which may act as antiholin
- The number of effective codons (Nc value) of TM4 lysin genes are lower than D29. However, the holin genes of D29 have lower Nc values than TM4. These results suggest that holin of D29 has better translational efficiency

- ♦ TM4 encodes no tRNA genes, whereas D29 has five tRNA genes that match with the highly used codons
- ♦ The residues surrounding the active site in TM4 lysin B is different from the well conserved residues of D29. Aspartic acid and histidine in D29 are replaced by glycine and alanine respectively in TM4

The holin gene of TM4 functioning as antiholin, its low translational efficiency and the lack of tRNA genes may be the reasons for D29 outperforming TM4 in lytic activity. Though codon usage analysis of whole genome indicates the overall expression level, further analysis of specific genes is required to understand their performance level.

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Ongoing studies

Antimycobacterial compounds from marine actinomycetes

(Collaboration with Indian Institute of Technology, Chennai and Periyar University, Salem)

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

Background

There is an urgent need to discover novel bioactive compounds to fight against drug-resistant *M. tuberculosis*. Actinomycetes are biotechnologically valuable prokaryotes which are a major source of antibiotics.

Aim

- To screen marine actinomycetes for antimycobacterial activity

Methods

Actinomycetes strains isolated from different marine ecosystems were screened for antimycobacterial and antibacterial activity. The CFs and mycelial methanol extracts of actinomycetes strains were tested against *M. tuberculosis* H_{37}Rv, a drug-resistant and a drug sensitive clinical isolate of *M. tuberculosis* by LRP assay. CFs of selected actinomycetes strains were extracted using different

solvents and tested for antimycobacterial activity. Ethyl acetate extract of the potential strain was purified by thin layer chromatography and bioassay guided fractionation. Spectral analysis of the active fraction was carried out at the Indian Institute of Technology, Chennai. Antibacterial activity was also studied by cross streak method and agar well diffusion method against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*.

Results

A total of 55 actinomycete strains were selected from different marine ecosystems, several of which exhibited antimycobacterial activity (table 16). Based on the results, 8 potential actinomycete strains were selected for further investigations. In bioassay guided fractionation, fraction II of ethyl acetate extract of R2 strain showed antimycobacterial activity. A number of actinomycete strains also showed activity against *S. aureus, B. subtilis, E. coli, S. typhin* and *P. aeruginose* by both cross streak and agar diffusion methods (Figs. 1 & 2).

Conclusion

Marine actinomycetes are potential sources for antimycobacterial compounds, since, 40 out of 55 strains tested inhibited *M. tuberculosis.* Purification, characterization and structure elucidation of the active compound from potential actinomycete strains are in progress.

Table 16: Number of actinomycete CFs and mycelial methanol extracts showing different range of antimycobacterial activity in LRP assay

Ranges of % RLU reduction	Culture filtrates			Methanol extracts		
	$H_{37}Rv$	SHRE-sensitive MTB	SHRE-resistant MTB	$H_{37}Rv$	SHRE-sensitive MTB	SHRE-resistant MTB
>50%	40	30	26	21	22	24
>50-75%	19	8	4	10	6	9
>75-90%	10	8	3	8	8	7
>90%	11	14	19	3	7	8
<50%	15	25	29	34	33	31

RLU – Relative light units; S – streptomycin; H – isoniazid; R – rifampicin; E – ethambutol; MTB – *M. tuberculosis*

Fig.1: Antibacterial activity of marine actinomycetes by cross streak method

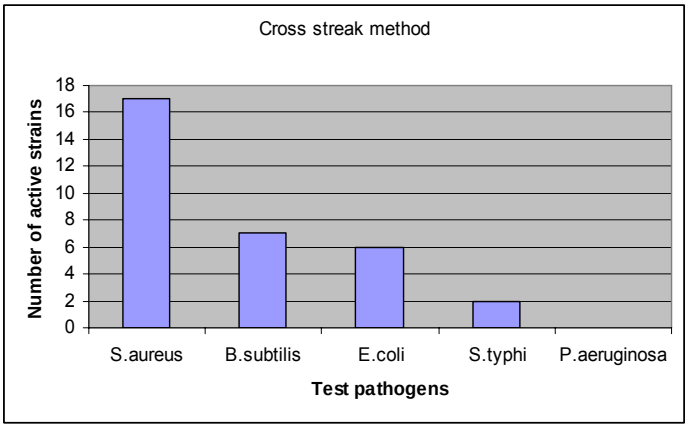
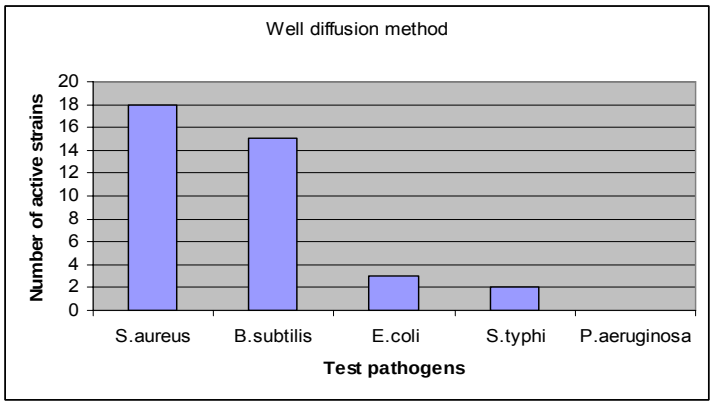


Fig. 2: Antibacterial activity of marine actinomycetes by well diffusion method



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Efficacy of slide culture in rapid detection of *M. tuberculosis* in sputum specimens processed by modified Petroff's method

Background

Rapid diagnosis of TB is vital for control and prevention of spread of the disease in the community. Assays aimed at field level applications should be simple, rapid, reliable not requiring expensive instruments. Slide culture technique is simple with high potential for rapid diagnosis and detection of drug-resistant strains directly from sputum specimens.

Aim

- To standardize slide culture technique for rapid detection of *M. tuberculosis* in sputum specimens processed by modified Petroff's method

Method

Fifty smear positive sputum specimens were included in the pilot study. The specimens were randomized and processed by modified Petroff's method. From the final pellet, two LJ slopes were inoculated and incubated. From the remaining deposit, triplicate smears each covering an approximate area of 0.05 cm^2 were made on one end of specially prepared sterile slides using a standard 5 mm twisted wire loop. The smears were air dried and gently heat fixed by

passing the smear thrice over the flame. Two smears were placed back to back in a universal container with 4 ml of Kirschner's liquid medium and incubated. After 7 days, the slides were removed, de-contaminated using Cidex, air dried and heat fixed. All three smears including the deposit smear were stained by auramine phenol. Growth on the incubated smears was compared with the deposit smear and graded. Results were compared with those obtained by the conventional culture method.

Results

Among the 50 samples that were included, three cultures were contaminated and were excluded. Among the remaining 47 samples, 40 were culture positive and 7 were culture negative. Among the 40 culture positives, 38 were positive by slide culture. Among the 7 culture negatives, 2 were negative by slide culture.

Conclusion

Slide culture technique shows promise in rapid detection of *M. tuberculosis* in sputum specimens. However, further work with larger number of samples has to be done to eliminate false results.

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Characterization of ethionamide resistance in naïve and treated TB patients from south India

Background

Ethionamide (ETH) is an efficacious, relatively non-toxic, second line anti-TB drug acting on the fatty acid synthesis of cell wall components of *M. tuberculosis*. Though ETH is a structural analogue of INH, only minimal cross resistance to these drugs is observed among clinical isolates. Although the activation of INH and ETH differs, the putative final metabolites for both drugs are very similar, and they share the same cellular target, namely inhA. Overproduction of the drug target also appears to lead to resistance to INH and ETH. ethA encodes a protein that belongs to the flavin containing monooxygenase family catalyzing the activation of ETH. ethR encodes a repressor belonging to the TetR/CamR family

of transcriptional regulators and negatively regulates the expression of ethA. The genetic locus, inhA has been known to be associated with resistance of *M. tuberculosis* to INH and ETH. Co-resistance to INH and ETH is not only mediated by dominant mutations in the target gene inhA, encoding an enoyl-ACP reductase, but also by recessive mutations in ndh, encoding a type II NADH dehydrogenase. Studies have shown no association between katG mutation and the level of ETH resistance, but mutations within the ethA and inhA structural genes were associated with relatively high levels of ETH resistance.

Rationale of the study

Researchers have carried out studies pertaining to ETH resistance and ETH and INH cross-resistance across the globe. But similar studies have not been carried out in a TB endemic setting like India. Clinical studies carried out at TRC have shown differences in the *in vitro* and *in vivo* activities of ETH. Screening TB patients for the presence of mutations / polymorphisms in the genes associated with ETH resistance might throw light on the level of primary resistance or possible acquired resistance within the geographical locale.

Aims

- To characterize the phenotypic and genotypic resistance pattern of ETH resistance among TB patients from south India
- To study the mechanism of cross resistance between INH and ETH by analyzing the genes conferring resistance to the drugs

Preliminary work

Standardization of phenotypic drug susceptibility testing for ETH was carried out on solid as well as automated liquid culture systems. Initial Phase I standardization was performed using *M. tuberculosis* H_{37}Rv and a panel of 15 *M. tuberculosis* strains isolated from naïve patients. Seventy *M. tuberculosis* strains (MDR and non-MDR) were included in phase II standardization. The results of the above standardization experiments and the retrospective analysis (n=407) for ETH suggest that MIC method is comparable with the proportion sensitivity

method which is the gold standard. Comparison of DST in solid medium with that of automated liquid culture systems will help in identifying the method that can best demarcate a sensitive strain from that of a resistant one.

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

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

Background

Concomitant RMP - based antitubercular therapy is known to significantly reduce NVP concentrations to sub-therapeutic levels in a significant proportion of patients, while in the case of EFV, the impact is to a relatively lesser extent. A few studies have observed that NVP-based ART has failed to yield satisfying clinical outcomes, compared to EFV-containing regimen in HIV-TB patients receiving treatment for both infections. However, it is not clear whether sub-therapeutic blood levels of NVP contribute to poor treatment outcomes in HIV-TB co-infected patients. In an ongoing controlled clinical trial at the Centre, two different ART regimens along with RMP-containing ATT are being evaluated in patients with HIV-1 and TB. The two regimens have either EFV or NVP with didanosine and lamivudine.

Aim

- To study the trough levels of EFV and NVP at different time points (during and after completion of ATT) and correlate with treatment outcome (viral load and CD4 cell count measurements)

Methods

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

So far, 64 and 105 patients receiving NVP and EFV respectively have been recruited into this study. The study is in progress.

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

ICMR-BIOMEDICAL INFORMATICS RESEARCH

Completed studies

In silico analysis of isoniazid resistance in *M. tuberculosis*

Background

Altered drug binding may be an important factor in INH resistance, rather than major changes in the activity of catalase or peroxidase. The identification of the structural or functional defects in the mutant enzymes remain under explored.

Aim

- To analyse INH resistance by molecular modeling and docking

Results

In this study the differences in the binding affinity between wild-type (WT) and mutants of KatG were investigated. In this process, five mutants of KatG (Asn138Ser [N138S], Ser315Thr [S315T], Ser315Asn [S315N], Ser315Iso [S315I], Ser315Arg [S315R] and a WT [S315]) were generated by the Modeller and the mutants were docked with INH by using the software-GOLD. The heme binding score suggested that categorization of the mutants would be in the order of S315N> N138S> S315R ≥ S315T >S315I /S315I< S315T ≤ S315R< N138S <S315N in imparting resistance. INH binding score suggested that the KatG mutant models, except N138S mutant may lead to least resistance, and probably may not be associated with any resistance. These models provide the first *in silico* evidence for the binding interaction of KatG with INH and implicate the basis for rationalization of INH resistance in naturally occurring KatG mutant strains of *M. tuberculosis*.

[Contact person: Dr.N.Selvakumar (E-Mail ID: selvakumarn@trcchennai.in)]

Protein - Protein interaction of heme oxygenase-1 and p38 mitogen activated protein kinase

Introduction

Chronic kidney disease, also known as chronic renal disease, is a progressive loss of renal function over a period of months or years through five stages. Heme Oxygenase-1 (HO-1) is the rate-limiting enzyme in the catabolism of heme. To date, the mechanism by which HO-1 functions as a cytoprotective and anti-inflammatory protein remains poorly understood. Induction of HO-1 has been found to have an adaptive and beneficial response to acute renal injury secondary to ischemia-reperfusion injury, nephrotoxins, glomerulonephritis, renal transplant rejection and rhabdomyolysis, *in vitro*. Members of the p38 family of mitogen activated protein kinases (MAPK) are primarily activated by stress stimuli, but are also activated during engagement of various cytokine receptors by their ligands. A previous study reported an increase of HO-1 mRNA and protein expression by TGF-β1 in human retinal pigment epithelial cells via p38 MAPK, and suggested that induction of HO-1 attenuates the adverse effects of elevated TGF-β1.

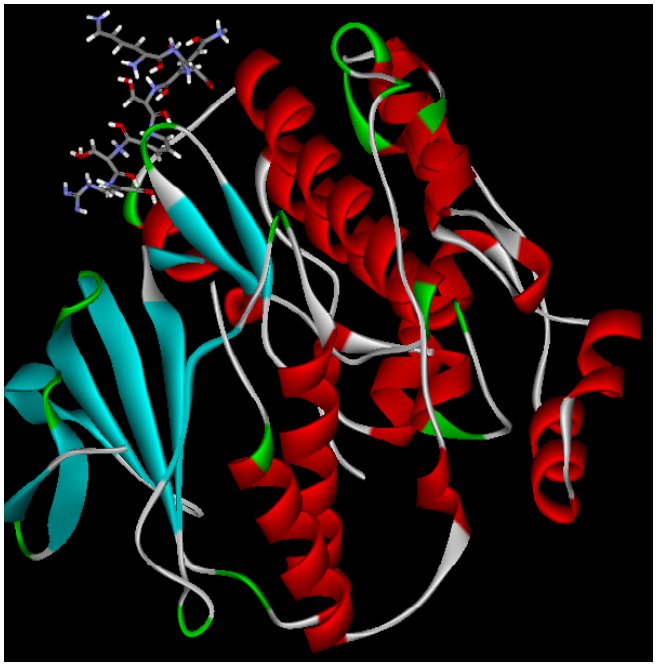
Aim

- To predict the interaction of heme oxygenase-1 and p38 MAPK

Results

In the present investigation, patients with renal failure showed enhanced HO-1 expression *ex vivo*. The study hypothesized that HO-1 might have adverse effects in renal failure cases. Evidence implicates its role in fibroblast formation, which might be one of the pathways for TGF-β mediated fibrogenesis via HO-1 upregulation. Previous reports claimed that p38 MAPK signalling molecule interacts with HO-1 at the transcription level. Also, the wet lab studies showed increased expression of p38 MAPK in the patient group. In order to have a better understanding of the surface interactions between HO-1 and p38 MAPK, *in silico* studies were premeditated. The binding site of HO1 was predicted to be between 226 and 232 amino acids. Protein protein docking between HO1 and p38 MAPK was done using CDOCKER (Fig. 3).

Fig. 3: HO-1 docked into the binding site of p38 MAPK



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

Molecular modeling and docking studies of PknL

Background

PknL, a eukaryotic like serine threonine protein kinase from *M. tuberculosis*, is predicted to be involved in transcriptional regulation and cell division.

Aim

- To predict the three dimenmsional structure of PknL

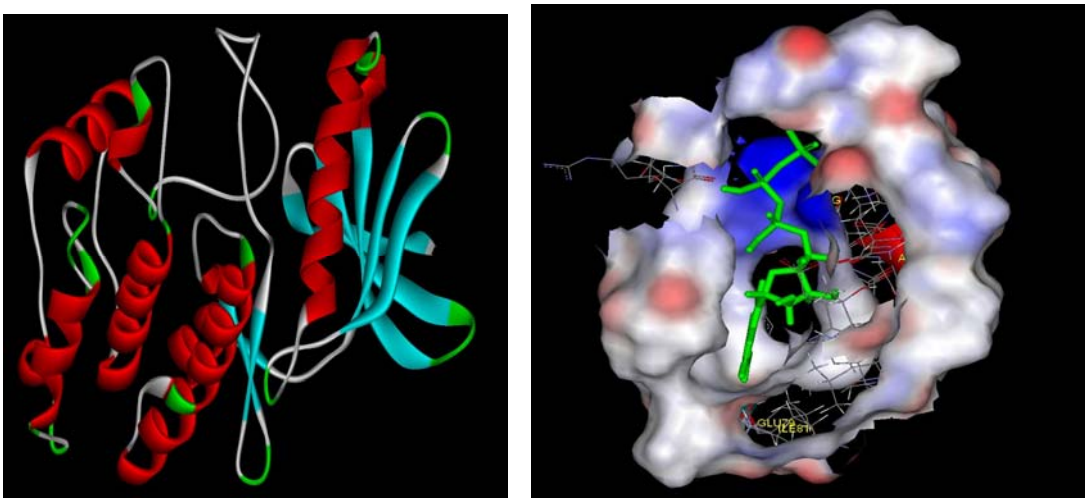
Results

In order to understand the conformational changes on binding to the ligand, the three dimensional models for apo and holo forms of PknL were predicted using two different templates (Fig. 4A). The kinase domain of PknE from *M. tuberculosis* (PDB ID 2H34) and the activation loop region of cAMP dependent protein kinase from *Saccharomyces cerevisiae* (PDB ID 1fot) was used as the structural template for building the apo structure of PknL domain. For developing a model for adenosine triphosphate (ATP) bound conformation for PknL, the

kinase domain of PknB from *M. tuberculosis* (PDB ID 106Y) and the activation loop region of phosphorylase kinase from rabbit (PDB ID 1q16) was used as the structural template. The results showed that the homology models were very similar to the template structures. Further, the ATP molecule was docked into the active site of holo model of PknL protein using CDOCKER (Fig. 4B).

Fig. 4:

A) Three dimensional model of PknL protein B) ATP docked into the active site of PknL protein using CDOCKER



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Ongoing studies

Target database for drug-resistant pathogens

Emergence of drug resistance is a major threat to public health. Morbidity and mortality are higher in infections caused by resistant pathogens than those caused by susceptible ones. Drug resistance has been reported in many infectious diseases across different countries, and multi DR forms of disease are extremely difficult to treat. MDR and extensively drug-resistant TB (XDR-TB) has been reported in most parts of the world. Similarly, strains of *P. falciparum* resistant to almost all of the available drugs have evolved. Hence, there is a need to develop novel drugs for the re-emerging DR diseases.

The target database for DR pathogens (TDDRP) contains information about all current drug targets and a list of potential targets, information on metabolic pathways involving the target genes and information on drugs used for each disease. The following pathogens are being included in the database during the first phase of the project: *M. tuberculosis, M. leprae, P. falciparum, P. vivax, S. aureus, S. pneumonia* and *N. gonorrohoea.* This database will be a useful resource for further research in drug discovery against drug-resistant infectious diseases. Work has been completed for *M. tuberculosis, M. leprae* and *P. falciparum.*

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

Database for drug-resistant tuberculosis

Two billion people, equal to one-third of the world's total population, are infected with *M. tuberculosis*, the microbe that causes TB. TB kills more than 2 million people per year and is a leading cause of mortality due to infectious diseases. The increasing emergence of drug-resistant TB, especially MDR-TB (resistant to at least two frontline drugs such as INH and RMP), is particularly alarming. MDR-TB has already caused several fatal outbreaks and poses a significant threat to the treatment and control of the disease in some parts of the world, where the incidence of MDR-TB can be as high as 14%. XDR-TB occurs when resistance to second-line drugs develop; this is extremely difficult to treat, and

cases have been confirmed in all regions of the world. The rise in the prevalence and death of TB cases is also due to the coinfection of TB patients with HIV. The lethal combination of DR-TB and HIV infection is a growing problem that presents serious challenges to effective TB control. Therefore, novel drugs effective against XDR-TB need to be developed. Besides, there is also a need to prevent the emergence of MDR-TB and XDR-TB and to manage the disease. To address these issues we are developing a database for drug-resistant TB named as DDR-TB.

The DDR-TB will contain clinical information on MDR-TB and XDR-TB. The patient information will be arranged under the following heads: Basic assessment form, monthly progress report, X-ray findings, haematology results, biochemistry results, urine analysis report, drug sensitivity test report, number of missed doses, etc. The effective control of drug-resistant TB requires effective public health infrastructure that will rapidly recognize and respond to it. This database is intended to serve this purpose. Further, the database will serve as a useful tool for identifying the changing pattern of the disease, besides being an online education tool to educate medical graduates.

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

BASIC RESEARCH

Completed studies

Studies on plasma vitamin D_3 level and the modulatory effect of vitamin D_3 on vitamin D receptor expression and intracellular cytokine positive T-cell subsets in pulmonary tuberculosis

Background

Our earlier studies revealed that vitamin D receptor (VDR) gene variants regulate vitamin D_3 modulated immune functions in normal healthy subjects (NHS) and PTB patients. Studying the role of variant genotypes of VDR on plasma 1,25 dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) level, VDR expression and intracellular cytokine positive cells will help to explore the basic molecular events associated with 1,25$(OH)_2D_3$ and immunity to TB.

Aim

- To study the regulatory role of variant VDR genotypes on plasma 1,25$(OH)_2D_3$ level, VDR expression and intracellular TNF-α and IFN-γ positive cells in PTB

Methods

The study subjects consisted of 75 PTB patients and 70 NHS. Estimation of plasma 1,25$(OH)_2D_3$ was undertaken using commercial ELISA kit. A portion of peripheral blood mononuclear cells (PBMC) was utilized for the estimation of basal VDR protein level using ELISA kit and in rest of the PBMC, a 48 hr culture was set up with live *M.tuberculosis* to enumerate the T-cell subsets positive for TNF-α and IFN-γ cytokines by flow cytometry.

Results

Plasma 1,25$(OH)_2D_3$ levels were found to be significantly increased among PTB patients compared to NHS ($p=0.008$) (Fig. 5). PTB patients had significantly lower levels of VDR protein compared to NHS ($p=0.009$) (Fig. 6). In NHS and PTB patients, a significantly reduced percentage of TNF-α and IFN-γ expressing CD3+ T-cells were observed in cultures stimulated with live *M.tuberculosis* and treated with 1,25$(OH)_2D_3$ compared to cultures without 1,25$(OH)_2D_3$ (NHS and PTB; CD3+TNF-α +: $p = 0.0001$; CD3+ IFN-γ+: $p = 0.0001$) (Figs. 7 & 8).

Fig. 5: Plasma $1,25(OH)_2D_3$ levels in NHS and PTB patients

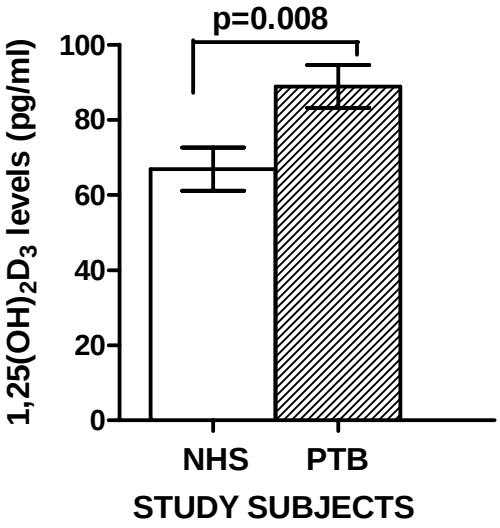


Fig. 6: Basal vitamin D receptor protein level in NHS and PTB patients

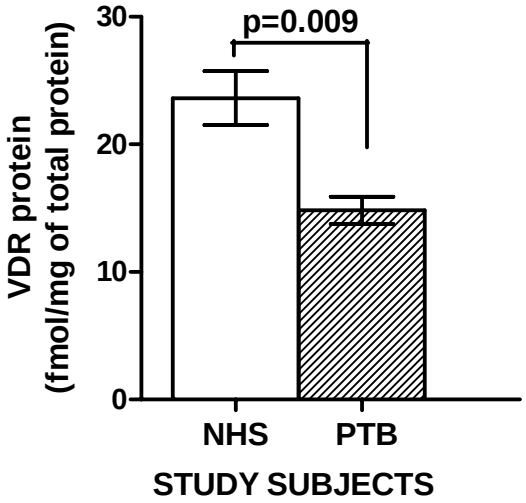
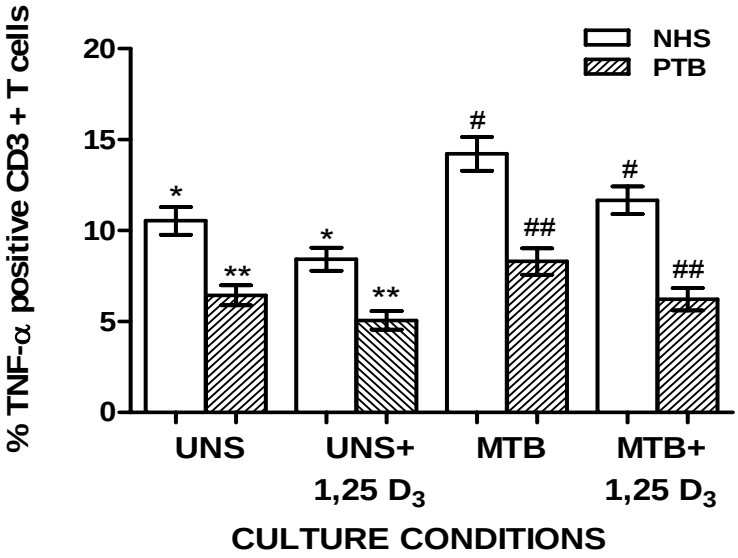
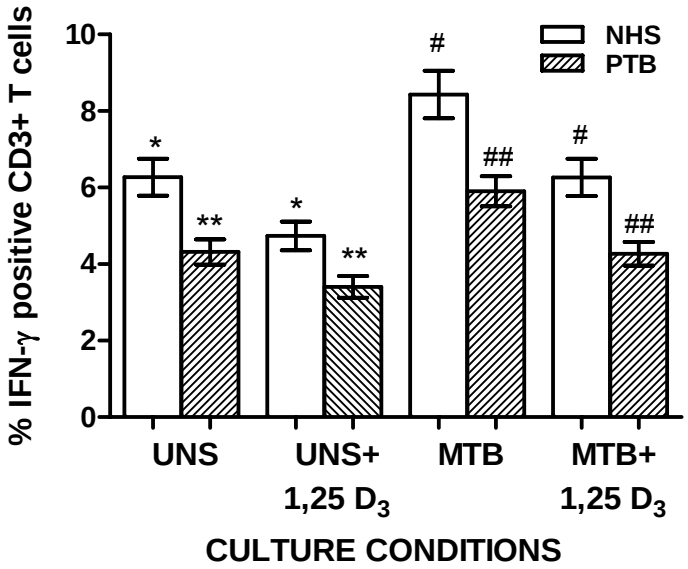


Fig. 7: Effect of $1,25(OH)_2D_3$ on TNF-α expression by CD3+T-cells in NHS and PTB patients to MTB stimulation



UNS represents unstimulated cells. *, **, #, ## p=0.0001.

Fig. 8: Effect of $1,25(OH)_2D_3$ on IFN-γ expression by CD3+T-cells in NHS and PTB patients to MTB stimulation



UNS represents unstimulated cells. *, **, #, ## p=0.0001.

VDR gene polymorphisms in the 5' regulatory (Cdx2), coding (FokI) and 3' untranslated region (BsmI, ApaI and TaqI) were analysed for their influence on

plasma 1,25$(OH)_2D_3$ levels. There were no significant changes observed in the 1,25$(OH)_2D_3$ levels among the VDR genotypes of PTB patients and NHS. With respect to the regulatory role of VDR gene variants on *ex vivo* VDR protein expression, patients with GG genotype of Cdx2 polymorphism had significantly increased levels of VDR protein compared to patients with AA genotype (p=0.021). However, no such difference was observed among NHS. VDR genotypes did not influence the expression of TNF-α and IFN-γ both in patients and NHS.

Conclusion

The results suggest that PTB patients have increased plasma 1,25$(OH)_2D_3$ levels and decreased expression of *ex vivo* levels of VDR protein in PBMC. Expression of VDR protein levels are influenced by the variants of Cdx2 polymorphism. Increased 1,25$(OH)_2D_3$ levels might lead to downregulation of VDR and could cause defective VDR signalling which may influence the down stream process that triggers innate immunity against *M. tuberculosis* infection. The study also revealed the suppressive effect of 1,25$(OH)_2D_3$ on single cell expression of TNF-α and IFN-γ by CD3+ T-cells in PTB. This suppressive effect of 1,25$(OH)_2D_3$ on proinflammatory and Th1 cytokine positive cells might have a role in reducing inflammation at the site of infection.

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

Effect of 1,25 dihydroxyvitamin D_3 on the expression of VDR, cathelicidin and CYP27B1 mRNA expression in pulmonary tuberculosis

Background

1, 25$(OH)_2D_3$, the active metabolite of vitamin D, exerts its effect through VDR and is known for its potent immunomodulatory activities. 1, 25$(OH)_2D_3$ has been shown to induce the expression of antimicrobial peptide cathelicidin and is associated with restricted growth of *M. tuberculosis* in monocytes under *in vitro* culture conditions. Using quantitative real-time polymerase chain reaction (RT-PCR), induction of VDR, cathelicidin and CYP27B1 (1α hydroxylase, an enzyme involved in converting 25, hydroxyvitamin D_2 to 1,25$(OH)_2D_3$) mRNA were studied

in live *M. tuberculosis* stimulated macrophage cultures treated with or without $1,25(OH)_2D_3$ in NHS and PTB patients.

Aim

- To study the effect of 1,25 dihydroxyvitamin D_3 on the expression of VDR, cathelicidin and CYP27B1 mRNA expression in PTB

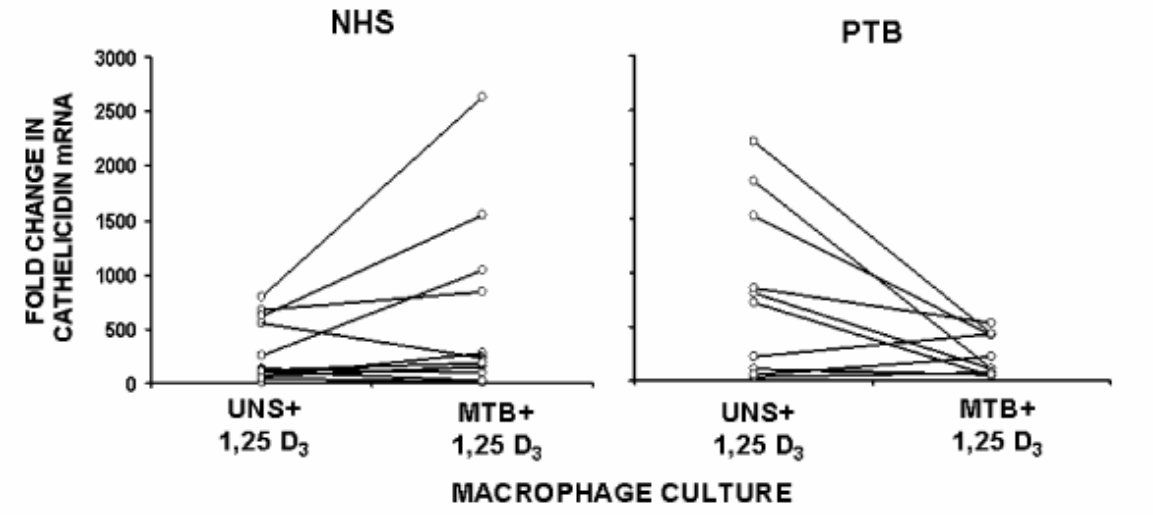
Methods

The study subjects consisted of 12 PTB patients and 10 NHS. Using quantitative RT-PCR, induction of VDR, cathelicidin and CYP27B1 mRNA were studied in live *M. tuberculosis* stimulated macrophage cultures treated with or without $1,25(OH)_2D_3$ in NHS and PTB patients.

Results

In PTB patients, expression of VDR mRNA was significantly higher in *M. tuberculosis* stimulated macrophage cultures compared to cultures with $1,25(OH)_2D_3$ ($p<0.05$) alone or with $1,25(OH)_2D_3$ and *M. tuberculosis* ($p<0.01$). When the fold induction levels were compared between NHS and PTB patients, PTB patients had relatively increased expression of VDR mRNA in 1, $25(OH)_2D_3$ treated cultures ($p<0.05$) or in cultures stimulated with *M. tuberculosis* ($p<0.05$). In both NHS and patients, stimulation with *M. tuberculosis* had no effect on the expression of cathelicidin mRNA. Interestingly, cultures treated with 1, $25(OH)_2D_3$ showed an increased expression of cathelicidin mRNA compared to unstimulated as well as *M. tuberculosis* stimulated cultures of both NHS ($p<0.05$) and PTB patients ($p<0.001$). In PTB patients, cathelicidin mRNA expression was higher in cultures with $1,25(OH)_2D_3$ alone compared to cultures with $1,25(OH)_2D_3$ and stimulated with *M. tuberculosis* ($p<0.05$). When cathelicidin mRNA expression was compared between NHS and PTB patients, cathelicidin mRNA expression was significantly higher in patients in cultures with 1, $25(OH)_2D_3$ alone ($p = 0.034$) (Fig. 9). Although CYP27B1 mRNA expression was not significantly different under different culture conditions compared to unstimulated cultures, a trend towards an increased expression of CYP27B1 was observed in cultures stimulated with *M. tuberculosis* in PTB patients.

Fig. 9: Relative expression of cathelicidin mRNA expression in macrophages stimulated with vitamin D_3 and *M. tuberculosis*



UNS: Unstimulated. In PTB, UNS+1,25 D_3 vs. MTB+1,25 D_3, $p<0.05$

Conclusion

The present study suggests that *M. tuberculosis* induced expression of VDR mRNA is higher in PTB patients. Vitamin D_3 enhances the expression of cathelicidin mRNA in PTB and NHS. VDR becomes functional upon exogenous addition of 1,25$(OH)_2D_3$ which might lead to increased expression of cathelicidin, an antimicrobial peptide associated with innate immunity in PTB.

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

Background

Our earlier study revealed the association of HLA -A11 with resistance and HLA -B40 and -DR2 with susceptibility to HIV and HIV-TB. Since variability among subtypes is known to influence HIV/AIDS differentially, identification of the subtypes of HIV/AIDS associated HLA antigens was attempted.

Aim

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

Methods

Molecular subtyping of HLA -A11, -B40 and -DR2 positive subjects among HIV patients with TB (HIV+TB+; n=104) and without TB (HIV+TB-; n=149), HIV negative TB patients (HIV-TB+; n=154) and healthy controls (n=168) was done using PCR based sequence specific oligonucleotide probe method and detection by chemiluminescence.

Results

Frequency of HLA -A*1101 was significantly lower while that of HLA -B*4006 was higher in overall HIV (HIV+TB- and HIV+TB+) and HIV+TB+ groups compared to healthy controls. Significant overrepresentation of HLA -DRB1*1501 in HIV+TB- patients and -DRB1*1502 in HIV+TB+ patients was observed as compared to healthy controls (table 17).

Table 17: Percent phenotype frequencies of selected HLA -A11, -B40 and -DR2 subtypes in HIV patient groups and healthy controls

HIV allele	Healthy controls n=168	Overall HIV n=253	HIV+TB- n=149	HIV+TB+ n=1041
A*1101	25.0[a,b]	12.2[a]	16.7	5.7[b]
B*4006	13.7[c,d,e]	30.0[c]	30.8[d]	28.8[e]
DRB1*1501	17.5[f]	27.0	33.0[f]	19.0
DRB1*1502	16.7[g]	23.0	19.4	28.0[g]

Note: n: number of subjects studied. P_c: p value corrected for the alleles studied.
[a]Overall HIV vs Healthy controls: p=0.001, P_c=0.012, OR 0.42 (95% C.I. 0.24-0.72).
[b]HIV+TB+ vs Healthy controls: p=0.0001, P_c=0.001, OR 0.18 (0.06-0.46).
[c] Overall HIV vs Healthy controls: p=0.0001, P_c=0.004, OR 2.71 (1.58-4.75).
[d]HIV+TB- vs Healthy controls: p=0.0003, P_c=0.008, OR 2.82 (1.56-5.17).
[e]HIV+TB+ vs Healthy controls: p=0.003, P_c=0.086, OR 2.56 (1.33-4.95).
For HLA -DRB1 typing, Healthy controls n=137; Overall HIV n=239; HIV+TB- n=139; HIV+TB+ n=100.
[f]HIV+TB- vs Healthy controls: p=0.004, P_c=0.06, OR 2.33 (1.28-4.29). [g]HIV+TB+ vs Healthy controls: p=0.010, P_c=0.15, OR 2.32 (1.20-4.53)

Conclusion

The results suggest a possible association of HLA -A*1101 (subtype of -A11) with resistance, while HLA -B*4006 (subtype of HLA -B40) with susceptibility to HIV and development of TB in HIV patients. Further, HLA -DR2 subtypes, viz HLA -DRB1*1501 might be associated with susceptibility to HIV-1 infection whereas HLA -DRB1*1502 may be associated with susceptibility to TB in HIV patients.

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

CD209, CCR2 and CCR5 gene polymorphisms in HIV and HIV-TB patients of south India

Background

CD209 gene encodes for a pattern recognition receptor, known to be exploited by both HIV-1 and *M. tuberculosis* for their survival. CCR2 and CCR5, the C-C chemokine receptors, act as co-receptors for HIV-1 entry into host cells. Polymorphisms in these genes may influence immune response against HIV/AIDS, and hence study of association of genetic variants of CD209, CCR2 and CCR5 with HIV and HIV-TB was attempted.

Aim

- To find whether polymorphisms in CD209, CCR2 and CCR5 genes are associated with susceptibility or resistance to HIV and HIV-TB

Methods

The study subjects comprised of 131 HIV positive TB negative patients (HIV+TB-), 107 HIV positive with PTB (HIV+TB+), 108 HIV negative TB positive patients (HIV-TB+) and 158 healthy controls. Genotyping of CD209 gene variants in the promoter region (-336 and -139), in the intron and 3' untranslated regions (In2+11 and 2281), CCR2 V64I and CCR5 polymorphisms were studied using PCR method.

Results

In CD209 -336 promoter polymorphism, significantly increased frequency of G/G genotype was observed in healthy controls and HIV+TB+ patients compared to

HIV+TB- patients (p=0.005; p=0.003 respectively) (table 18). The allele and genotype frequencies of CCR2 V64I polymorphism were not different between various patient groups and healthy controls. CCR5Δ32 polymorphism was observed in only one HIV-TB+ patient.

Table 18: Percent genotype frequencies of CD209 -336 polymorphism in healthy controls, HIV and HIV+TB+ patients

CD209 (-336) genotype	Healthy controls n = 158	HIV+TB- patients n=131	HIV+TB+ patients n=107	HIV-PTB+ patients n=108
A/A	62.4	64.1	70.1	60.8
A/G	30.6	35.9	23.4	35.5
G/G	7.0*	0*@	6.5@	3.7

* Controls vs HIV+TB- p= 0.005

@ HIV+TB- vs HIV+TB+ p= 0.003

Conclusion

The present study suggests that -336 G/G genotype may be associated with protection against HIV-1 infection. Moreover, the same genotype is associated with susceptibility to TB among HIV-infected patients. The study results also suggest that CCR2 V64I (G/A) and CCR5Δ32 polymorphisms are not associated with susceptibility or resistance to HIV and TB.

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Phenotypic modulations in infected neutrophils during tuberculosis

Background

Polymorphonuclear leucocytes (PMN) or neutrophils infiltrate to the inflammatory sites and phagocytose mycobacteria, thereby inhibiting the bacillary spread initially until macrophages accumulate and get activated.

Aim

- To study the interaction of neutrophils with prevalent clinical strains (S7 and S10) of *M.tuberculosis* and the subsequent morphological changes

Methodology

Dextran purified neutrophils from normal subjects and TB patients were infected with *M. tuberculosis* strains and cultured for a duration of 3 and 18hrs. At the end of termination, the cell surface expression of CD16, CD69, CXCR2 and induction of apoptosis were analyzed using flow cytometry. Cytokines and chemokines were assessed in supernatants by ELISA.

Results

All infected PMNs showed a decrease in CD16 at 18hrs in both groups. The expression of CD69 and CXCR2 was significantly higher at an early time point in TB-PMN compared to normals (Figs. 10A, B & C). Increased pro-inflammatory cytokine (TNF-α) and chemokine (IL-8) response was observed in infected neutrophils at 3hrs in both the groups (Figs. 11A & B). The strains infected neutrophils also showed high phagocytic indexes and increase in apoptosis in TB-PMN (tables 19 A & B).

Fig. 10(A): Expression profile of CD16

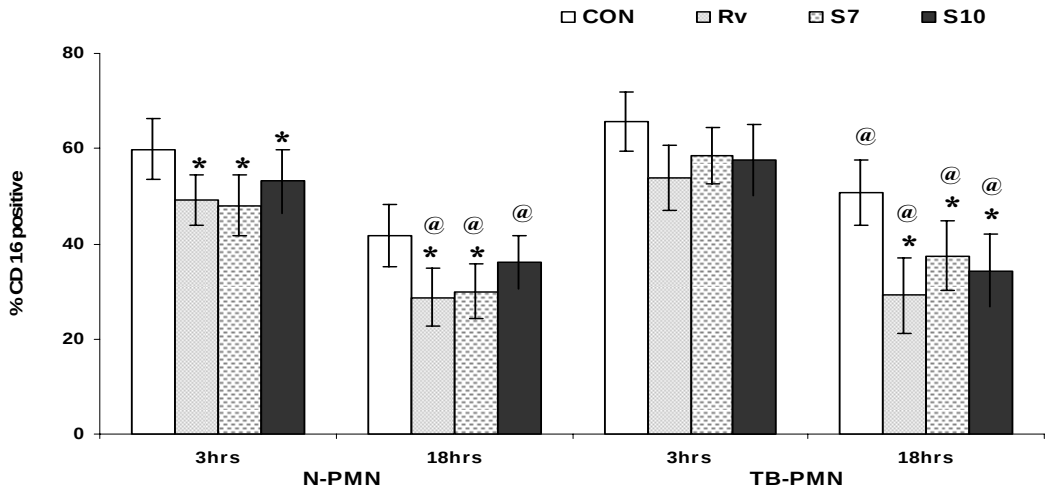


Fig. 10 (B): Expression profile of CD69

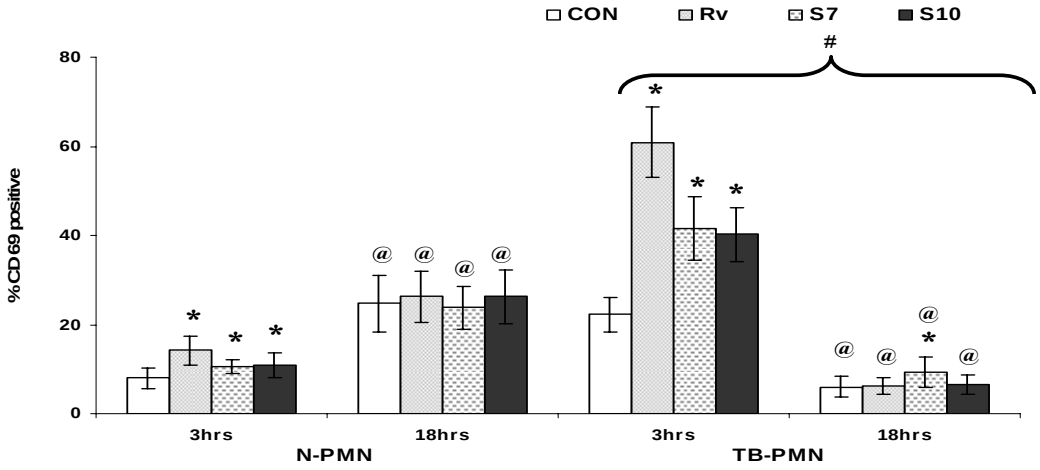
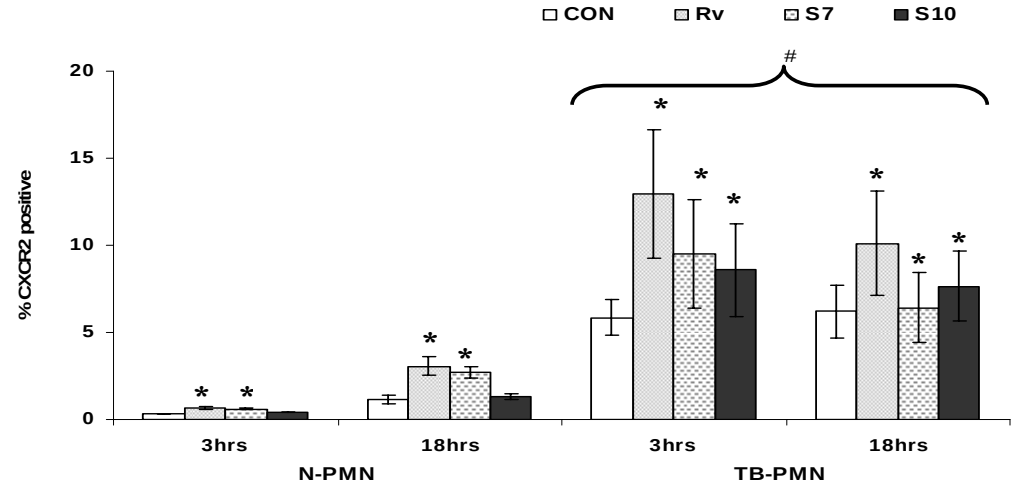


Fig. 10 (C): Expression profile of CXCR2



* Comparison with uninfected control, @ with respective 3hrs PMN and # with respective N-PMN

Fig. 11 (A): TNF-α levels

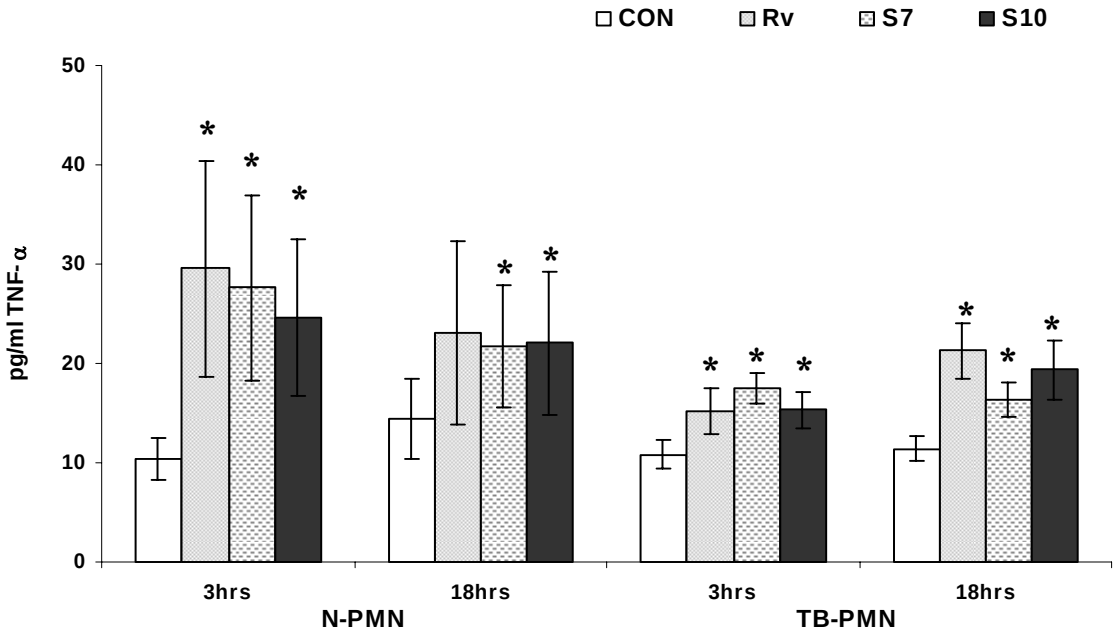
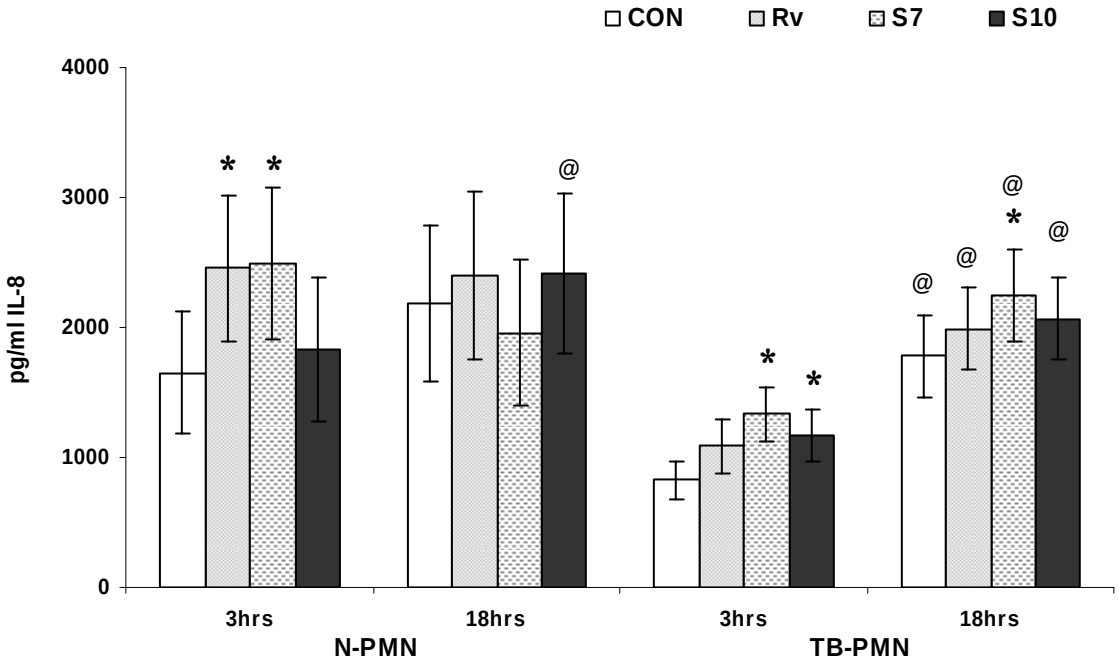


Fig. 11 (B): IL-8 levels



* comparison with uninfected control , @ comparison with respective 3hrs PMN

Table 19 (A): Phagocytic indices for various *M. tuberculosis* strains by N-PMN and TB-PMN

	N-PMN (n=15)		TB-PMN (n=15)	
	3 hrs	18 hrs	3 hrs	18 hrs
H37Rv	80.5 ±5.6	82.2 ±3.4	62.3 ±6.5	79.0 ±3.2 [b]
S7	149.7 ±33.8 [a]	87.8 ±3.2 [b]	90.8 ±5.4 [a]	82.4 ±3.5
S10	119.1 ±10.8 [a]	87.6 ±4.1 [b]	94.6 ±6.8 [a]	82.7 ±3.0 [b]

[a]*P*<0.05 compared with Rv , [b]*P*<0.05 compared with respective 3 hrs

Table 19 (B): Apoptosis of neutrophils induced by the *M. tuberculosis* strains

	N-PMN (n=20)		TB-PMN (n=20)	
	3 hrs	18 hrs	3 hrs	18 hrs
Control	13.4 ± 4.0	20.6 ± 5.8	21.5 ± 6.1	24.6 ± 6.2
H37Rv	19.6 ± 4.5*	25.6 ± 4.3*	26.65 ± 5.8*	29.2 ± 7.0*
S7	16.0 ± 4.9	26.0 ±4.6*	26.5 ± 6.3*	29.0 ± 5.8*
S10	17.0 ±5.1	26.2 ±5.2*	26.5 ± 5.1*	28.4 ± 7.0*

**P*<0.05 compared with control

Conclusion

In vitro infection studies demonstrated varying degrees of modulation of neutrophil functions in both the groups. TB-PMN was found to be more competent in amplifying the innate immune response and conferring protection at the early phase of infection. However, the response was not strain specific in either of these groups.

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Cytotoxic T-lymphocyte responses against HIV-1 C epitopes among HIV-1 infected south Indians

Background

The observation that recovery from many viral infections is followed by the development of lifelong immunity is the fundamental principle of vaccinology. In contrast, the fact that everyone who becomes infected with HIV remains persistently infected and ultimately develops disabling immunodeficiency has been a serious discouragement to HIV vaccine development. A strong Cytotoxic T-lymphocyte (CTL) response is often associated with better virus control and slow disease progression. CTL epitopes have been well defined for HIV-1 subtype B. However, only a limited number of studies have examined CTL epitopes in clade C HIV-1, although it accounts for >90% infections worldwide.

Aim

- To identify immunogenic T-cell epitopes in the Gag protein of HIV-1 subtype C commonly recognized in a cohort of HIV-infected south Indians

Methods

The study population comprised of 31 HIV-infected individuals who were participating in a controlled clinical trial for HIV at TRC, Chennai. Epitope mapping was performed using the HIV-1 gag peptide matrix (comprising of 22 overlapping peptide pools) ELISPOT technique. Study subjects were HLA typed by PCR. *In silico* tools were employed to predict probable epitopes in the immunogenic peptides using the Pro Ped software.

Results

Majority of the study participants were found to express HLA B*40 and HLA A*02 alleles. A total of 34 epitopic regions spread over the p17, p24 and nucleocapsid regions of the Gag protein elicited dominant CTL responses (table 20). Each of these regions in turn contained multiple epitopes. It was observed that many of the immunodominant epitopes identified in clade C HIV were conserved in HIV-1 clade B as well.

Conclusion

Three epitopic regions identified in this study are novel and have not been reported earlier. We found no correlation between CD4+ T-cell counts, number of gag pools recognized and number of peptide regions recognized, indicating that the disease state in individuals within this cohort was unrelated to the breadth and magnitude of T-cell epitope responses. This study adds to the existing knowledge bank of HIV-1 epitopes recognized by Indians, and will aid in the development of vaccine candidates for this population.

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

Table 20: Epitopic regions in HIV-1C Gag recognized by south Indians

HIV Antigens	Location	Sequence	No. of responders
p17	1-19	MGARASILRGGKLDKWEK	5
	17-43	EKIRLRPGGKKHYMLKHLVWASRELER	6
	25-55	GKKHYMLKHLVWASRELERFALNPGLLETSE	3
	45-67	ALNPGLLETSEGCKQIMKQLQPA	6
	61-79	MKQLQPALQTGTEELRSLY	6
	69-99	QTGTEELRSLYNTVATLYCVHEEIEVRDTKE	3
	73-87	EELRSLYNTVATLYC	5
	81-95	TVATLYCVHEEIEVR	2
	89-107	HEEIEVRDTKEALDKIEEE	5
p17-p24	109-135	NKSQQKTQQAKAADGKVSQNYPIVQNL	2
p24	137-203	GQMVHQAISPRTLNAWVKVIEEKAFSPEVIPMFTALSEGATPQDLNTMLNTVGGHQAAMQMLKDTIN	4
	149-175	LNAWVKVIEEKAFSPEVIPMFTALSEG	6
	157-171	EEKAFSPEVIPMFTA	5
	177-207	TPQDLNTMLNTVGGHQAAMQMLKDTINEEAA	5
	197-215	MLKDTINEEAAEWDRLHPV	3
	209-227	WDRLHPVHAGPIAPGQMRE	1
	217-231	AGPIAPGQMREPRGS	3
	221-243	APGQMREPRGSDIAGTTSTLQEQ	4
	233-263	IAGTTSTLQEQIAWMTSNPPVPVGDIYKRWI	3
	245-275	AWMTSNPPVPVGDIYKRWIILGLNKIVRMYS	2
	269-287	KIVRMYSPVSILDIKQGPK	6
	285-303	GPKEPFRDYVDRFFKTLRA	3
	297-351	FFKTLRAEQATQDVKNWMTDTLLVQNANPDCKTILRALGPGASLEEMMTACQGVG	2
	309-363	DVKNWMTDTLLVQNANPDCKTILRALGPGASLEEMMTACQGVGGPSHKARVLAEA	1
p24-NC	353-375	PSHKARVLAEAMSQANSTNIMMQ	3
NC	365-391	SQANSTNIMMQRSNFKGPKRIVKCFNC	2
	369-395	STNIMMQRSNFKGPKRIVKCFNCGKEG	4
	385-403	IVKCFNCGKEGHIARNCRA	1
	397-439	IARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSHKG	5
	409-431	CWKCGKEGHQMKDCTERQANFLG	3
	421-447	DCTERQANFLGKIWPSHKGRPGNFLQS	4
	437-467	HKGRPGNFLQSRPEPTAPPAESFRFEETTPA	2
	449-471	PEPTAPPAESFRFEETTPAPKQE	3
	461-492	FEETTPAPKQEPKDREPLTSLKSLFGSDPLSQ	1

Comparison of drug resistance pattern between proviral and plasma HIV-1

Background

It is believed that the rate of transmitted drug resistance is low in the naïve south Indian population, with a high rate of drug resistance mutations (DRM) of the virus seen only in patients failing ART (unpublished data). A few studies in the past have reported that DRMs are detected at different levels in DNA from PBMCs and in plasma RNA, possibly due to the different rates of turnover of the virus in the two compartments, and that there are discrepancies between DRMs seen in plasma RNA and PBMC DNA in subjects failing therapy as well as in those who have stopped treatment.

Aim

- To examine the sequence of HIV-1 proviral DNA obtained from patients failing treatment and from ART-naïve individuals, to determine whether any of them have archived DRMs which are not easily detected in the circulating virus population

Methods

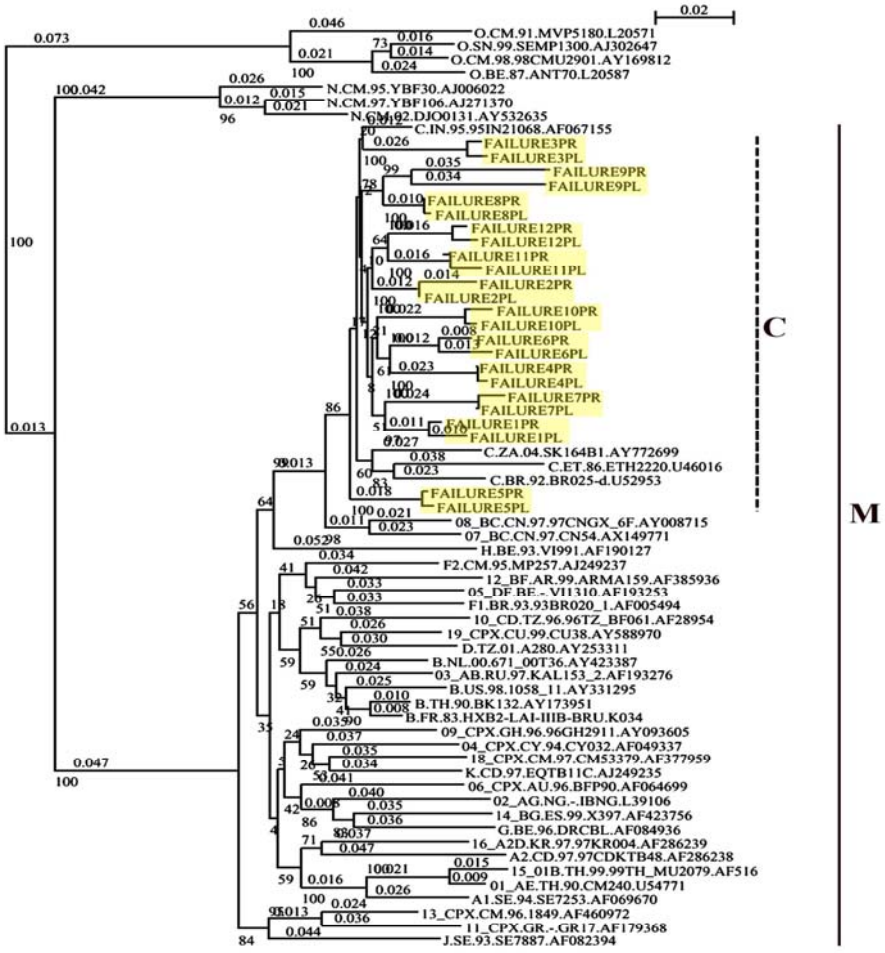
We analyzed DRMs and polymorphisms in the reverse transcriptase (RT) gene in proviral DNA as well as in the plasma HIV RNA in HIV-1 infected individuals. Paired plasma and whole blood samples were obtained from 18 HIV patients naïve to ART and 15 HIV patients treated with anti-retroviral drugs but failing treatment and subjected to DR testing using an in-house protocol. RT gene from samples obtained from both the compartments was PCR amplified successfully in 12 individuals.

Results

At least one major mutation to both classes of RT inhibitor drugs was detected in specimens obtained from both compartments in individuals who had failed ART (Fig.12). The NRTI mutations detected were L74V, M184V, TAMs T215I and K219E. The most common NNRTI mutation observed was Y181C, followed by K103N, G190A, K101E, V106IV and V106M. The agreement in the detection of DR mutations to NRTI and NNRTI in the two compartments was found to be good and moderate using the kappa test. Thymidine analog mutations (TAMs)

were identified in 2 patients, but it was present in either the proviral or the plasma specimen and not in both. In ART-naïve individuals, the M184I mutation that confers resistance to 3TC and two TAMs T215F and K219E were detected, one in each patient in either compartment, while the rest had no major DR mutations.

Fig. 12: Pattern of mutations to antiretroviral drugs



The strains from our center are highlighted and indicated as failures followed by numbers. PL and PR denotes the respective plasma and proviral DNA sequences.

Conclusion

Our observations suggest that plasma RNA remains the elective choice for HIV genotyping in the south Indian population, and in cases where plasma storage is difficult, proviral DNA can be used for genotyping. Alternatively, the very short duration of exposure to the drugs in our study population could be a possible reason for the high level of agreement seen between the proviral and plasma specimens. Further studies on samples exposed to drugs for more than a year would explain if the agreement will remain unaltered or whether more archived mutations would develop in due course.

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Validation of in-house drug resistance method

Background

ViroSeq kit version 2.0 is the most widely used reagent for HIV DR testing. However, the high cost of the kit encouraged us to develop an in-house method for genotypic DR testing for HIV using primers specifically designed for HIV-1 subtype C which is the predominant clade of HIV-1 circulating in India.

Aim

- To validate the in-house methodology against the Viroseq protocol

Methods

Plasma was obtained from 13 HIV-positive ART-naïve individuals and 7 HIV-positive ART-experienced individuals who had failed treatment. Sequences of the RT and protease genes of the HIV isolates obtained by the two methods were aligned using CLUSTAL W, and sequence similarity was assessed. A similarity score of 95-99% was obtained for all paired sequences analyzed.

Results

No major DRM was identified in the RT gene of any of the 13 ART-naïve patients using both methods. However, one of these patients had the L90M mutation, a major mutation conferring high level resistance to the protease inhibitor (PI), nelfanavir and intermediate to low level resistance to the other PIs, in the protease gene; this mutation was successfully detected by both the methods.

The 7 ART-experienced individuals who had failed treatment had several polymorphisms. While polymorphisms at positions 35, 60, 121, 135, 162, 177, 207, 214 and 245 present in all the specimens were detected with 100% agreement between the two methods, there was only 85 – 92% agreement between the two methods in detecting polymorphisms at positions 36, 39, 48, 173, 178, 200, 203 and 211 which were found to be present in about half the individuals. Table 21 lists the various DRMs identified in the study.

Conclusions

The in-house method developed at TRC was found to exhibit 93% agreement with the commercial kit in the identification of major DRMs, besides being specific for subtype C HIV-1 and also very cost effective (approximately one-third the cost of a Viroseq test). Further, the in-house protocol requires a much lesser sample volume when compared to the Viroseq protocol facilitating use of pediatric samples which are difficult to obtain. The in-house drug-resistant testing method was able to amplify plasma samples with a minimum viral load of 800 copies/ml.

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Table 21: Drug-resistant mutations detected by in-house and Viroseq methods

Sample No.	VIROSEQ		In-house		VIROSEQ			In-house				VIROSEQ	In-house
	NRTI				NNRTI							PIs	
1	L74V	M184V	L74V	M184V	K101KE	Y181C	G190A	K101KE	Y181C	G190AG	H221HY	None	None
								V108VI					
2		M184V		M184V	K103N	V106M	E138A	K103N	V106M	E138A		None	None
3	L74V	M184V	L74V	M184V	V108I	Y181C	H221Y		Y181C	H221Y		None	None
4	L74V	M184V	L74V	M184V	K103N	Y181C	H221HY	K103KN	V108VI	Y181C	H221HY	None	None
5	None		None		None			None				None	None
6	K65R	M184V	K65R	M184V	K101HQ	Y181C	G190A	K101H	Y181C	G190A		L33F	None
7	L74ILV	M184V	V75IMV	M184V	L100IL	K103N	V108IV	K103N	V108IV	P225HY	L234IL	None	None
	V75IMV				P225HY								

Ongoing studies

Effect of 1, 25 dihydroxyvitamin D_3 on matrix metalloproteinases in pulmonary tuberculosis

Background

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

Aim

- To study the effect of 1, 25 dihydroxyvitamin D_3 on MMPs, MMP-7, MMP-9 and the tissue inhibitor of metalloproteinases (TIMP)-1 in PTB

Methods

The study subjects consist of 20 PTB patients and 15 NHS. PBMC cultures were stimulated with CFA and live *M. tuberculosis* H37Rv in the presence and absence of 1, $25(OH)_2D_3$ for 48 hrs, and the culture supernatants were assayed for MMP-7, MMP-9 and TIMP-1 by commercially available ELISA kits (R&D systems, Minneapolis, MN, USA).

Results

A significant decrease in the spontaneous production of MMP-7 ($p=0.007$), increase in MMP-9 ($p=0.07$) and TIMP-1 ($p=0.0001$) was observed in PTB patients as compared to NHS. Vitamin D_3 significantly reduced the MMP-7 ($p=0.0001$) and MMP-9 ($p=0.0001$) and increased the TIMP-1 ($p=0.005$) level in antigen stimulated and unstimulated cultures of PTB as compared to NHS.

The study is in progress.

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

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

(Funded by Department of Biotechnology, New Delhi)

The aim of this project is to study the expression of HIV 1C gp41 epitopes on two self-assembly systems *viz*. the outer membrane porins of *Salmonella typhi* and the coat protein of cardamom mosaic virus (CMV). An epitope from HIV-gp41 from the Bangalore isolate of the virus has been engineered at the N-terminal part of CMV coat protein and also in the loop 7 of *S.typhi* OmpC. The chimeric proteins have been over-expressed, purified and presented as inclusion bodies. Reactivity of sera from HIV patients with the chimeric proteins have been analyzed by western blot.

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

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

Background

For the identification of the T-cell antigens, we had resolved culture filtrate proteins (CFP) of *M. tuberculosis* by using 2D-liquid phase electrophoresis (2D-LPE). Separated fractions were subjected to immunological analysis in TB patients and healthy contacts of TB patients. It was identified that 10 fractions were specifically recognized by contacts alone. Proteomic analysis revealed that 16 proteins were present in the 10 “contact specific” fractions. Among the 16 proteins, 13 were already reported as T-cell antigens in earlier studies and the remaining 3 (Adk, AcpM, Rv3716c) were novel T-cell antigens.

Aim

- To over express and purify the novel T-cell antigen, Adk, in an *E. coli* expression system

Methods

Recombinant plasmid containing Adk gene was transformed into *E. coli* BL21 (DE3) plys strain for over expression. The purification of Adk protein was done from the whole cell lysates, using immobilized metal affinity chromatography. Eluted fractions were checked by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

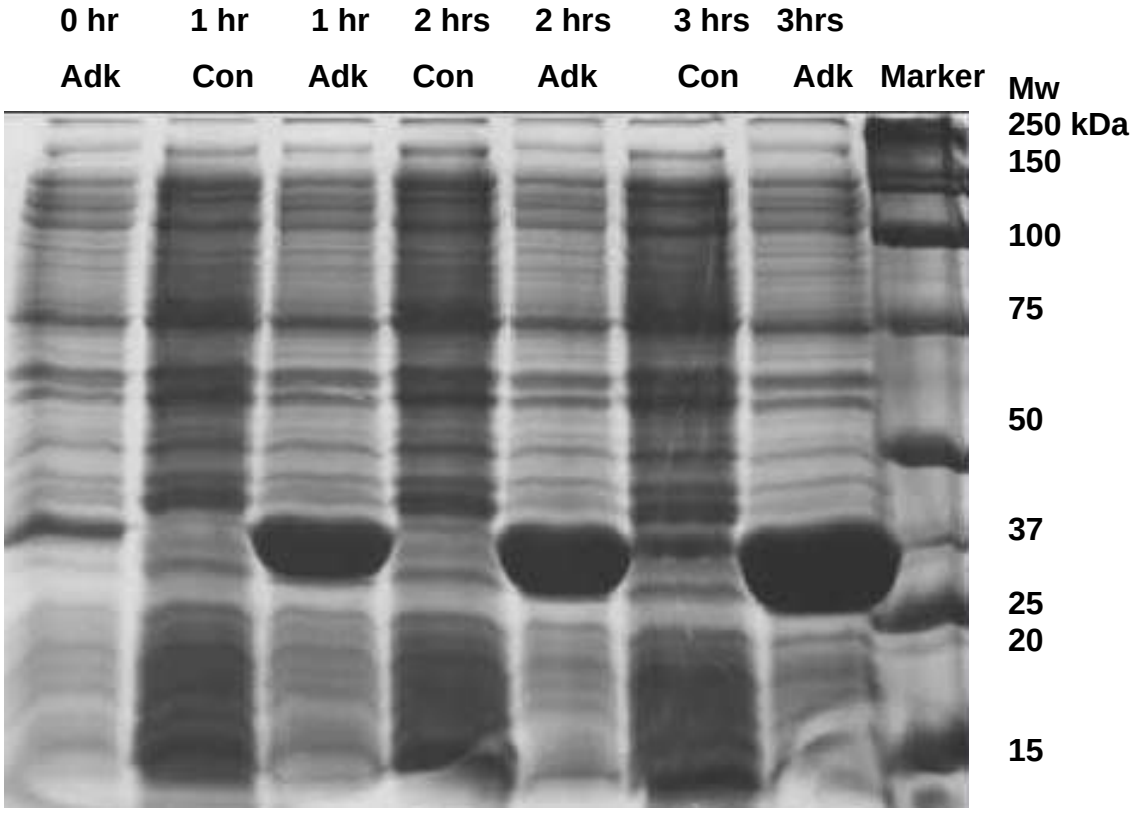
Results

BL 21 (DE3) plys transformants of Adk were induced with 1mM isopropyl thiogalactoside for the expression of recombinant protein with Histidine tag. Induction of the culture was carried out at different time points such as 1, 2, 3, 4, 5, 6 and 7 hrs and overnight. Levels of induction studied using SDS-PAGE showed that induction occurred as early as 1 hr and optimum high-level expression of the protein was found at 3 hrs at 37^{o}C (Fig. 13).

The recombinant protein was purified from soluble cytosolic fraction by nickel NTI affinity chromatography and the protein of interest was eluted using 50 mM imidazole. The fractions obtained were analyzed by SDS-PAGE (Fig. 14). The Adk protein containing fractions were pooled together and dialyzed to remove imidazole. For the immunological characterization of Adk protein, another set of TB patients and healthy house hold contacts (HHC) of patients were newly recruited (different from the 10 TB and HHC who were recruited for initial screening of antigens). This study is now ongoing.

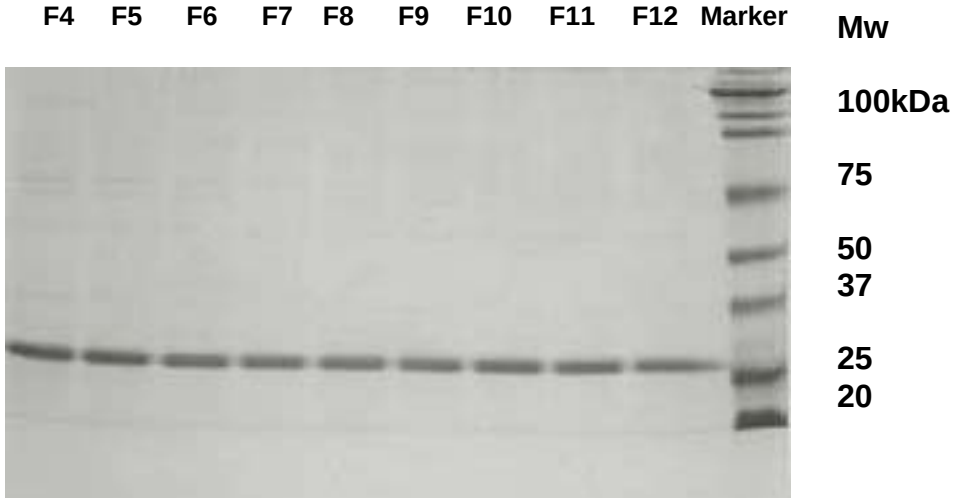
[Contact person: Dr. Alamelu Raja (E-mail ID: alamelur@trcchennai.in)]

Fig. 13: Time kinetics of Adk expression



Adk represents the lysates of cells transformed with recombinant adk gene containing plasmid.
Con represents the lysates of the cells transformed with control empty plasmid.
Marker represents the protein molecular weight marker.

Fig. 14: SDS-PAGE analysis of purified Adk protein fractions



F4 to F12 represent the fraction numbers which contained Adk protein.
Marker represents the protein molecular weight marker.

Innate immunity in HIV infection

Background

Tuberculosis, the most common opportunistic infection in HIV-positive subjects weakens the ability of the host to control infection. Chemokines play an important role in recruiting lymphocytes at the site of infection. Chemokines are small 8-10 kDa chemo-attractant molecules that are critical in recruitment of leukocytes to areas of inflammation and infection. Though the chemokines are secreted by various cell subsets, depression of adaptive immunity due to HIV infection in HIV-TB patients highlight the importance of natural killer (NK) cell mediated chemokine response.

Aim

- To investigate the role of chemokines secreted by NK cells in HIV infection particularly when co-infected with TB
- To study the chemokine response of NK cells upon stimulation with recombinant human cytokines

Methods

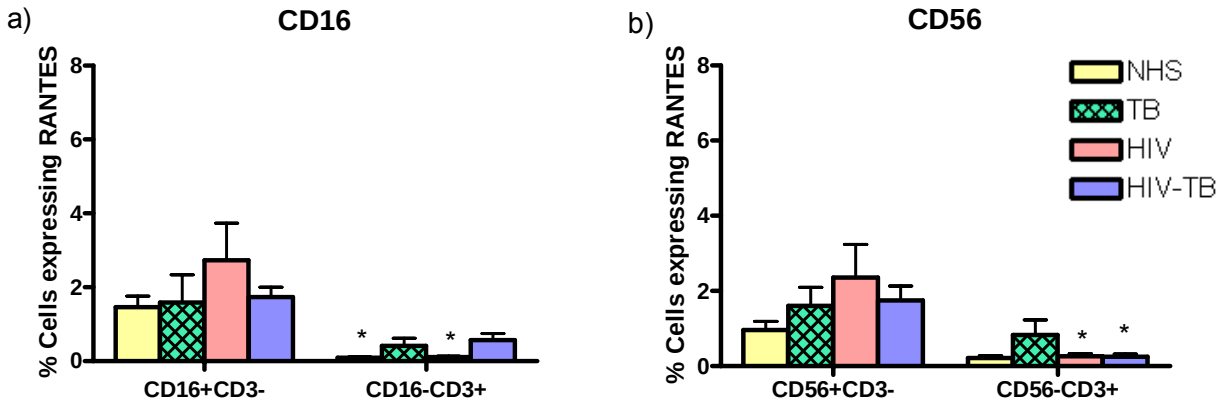
Study groups included NHS, patients with PTB, HIV-positive subjects without TB and HIV-positive patients with TB; each group had 10 subjects. Intracellular staining method was used to enumerate the chemokine positive CD16+ and CD56+ NK cells. The chemokines studied were RANTES, MIP-1α, MIP-1β and MCP-1.

Results

The cytotoxic activity and cytokine response of NK cells in NHS, TB, HIV and HIV-TB were reported in Annual Report 2007-08. The chemokine response of NK cells are presented in this report.

Basal expression of RANTES, MIP-1α and MIP-1β by CD16+ (immature) or CD56+ (mature) NK cells were elevated in HIV patients compared to NHS; however the increase was not significant. Basal RANTES, MIP-1α and MIP-1β expression by NK cells were augmented ($p<0.05$) in HIV and HIV-TB groups compared to CD3+ T-cells [Figs. 15 (a & b)].

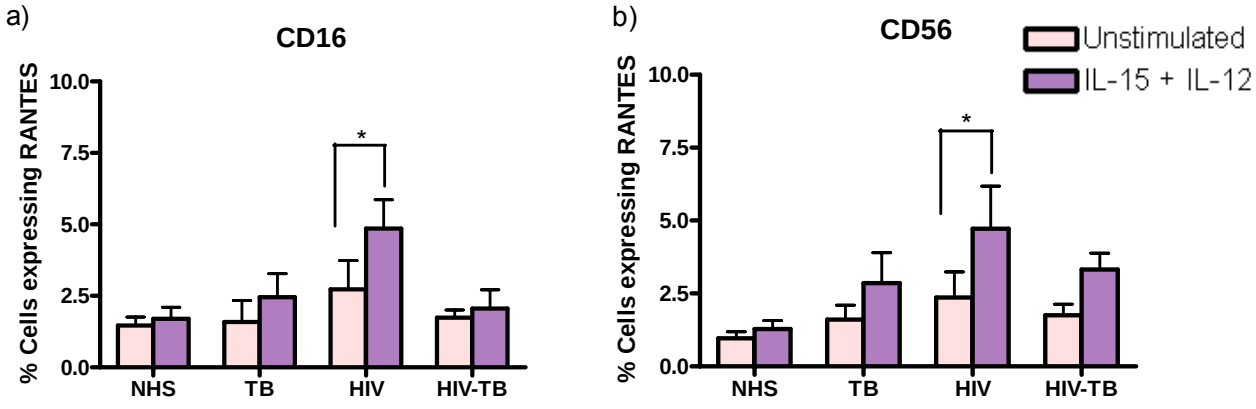
Figs. 15 (a & b): Basal expression of RANTES in lymphocytes



Data are presented as mean of 10 subjects in each group. Vertical bars denote standard error of mean. Statistical analysis was carried out using one-way ANOVA. *refers to the significance ($p<0.05$) where the comparisons are between NK cells and CD3+ cells

Stimulation with IL-15+IL-12 was found to improve the expression of RANTES, MIP-1α and MIP-1β by NK cells in the HIV group ($p<0.05$). Such an increase was not apparent in the other groups [(Figs. 16 (a & b)].

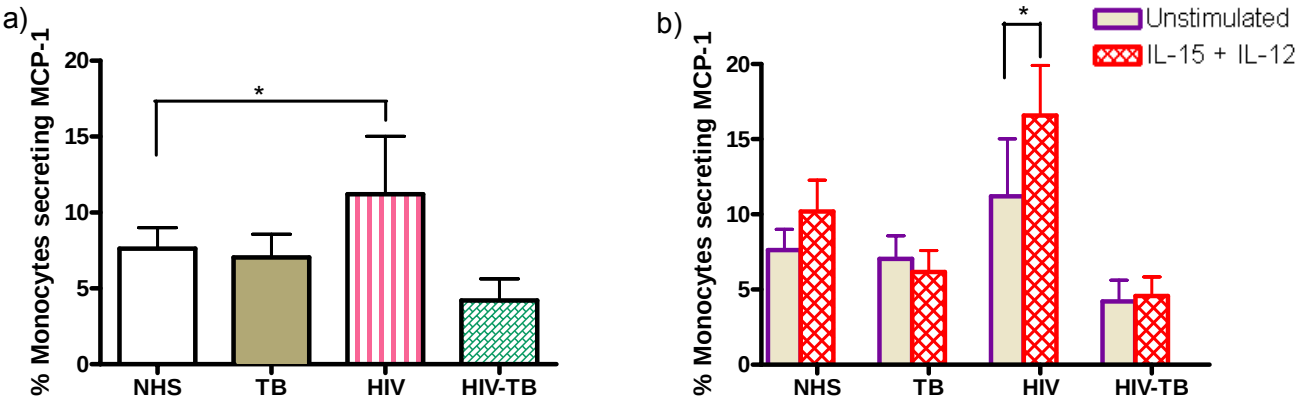
Figs. 16 (a) & (b): Effect of IL-15+IL-12 on the expression of RANTES in NK cell



Data are represented as mean of 10 subjects. Vertical bars denote standard error of mean. Statistical comparisons were done between unstimulated and IL-15+IL-12 stimulation using two-way ANOVA. *represent significant difference $p<0.05$

The frequency of monocytes expressing MCP-1 increased in HIV infection (p<0.05), irrespective of the presence or absence of stimulation [Figs. 17 (a) & (b)].

Figs. 17 (a) & (b): MCP-1 expression by monocytes before and after stimulation with IL-15 and IL-12



Values are given as mean of 10 subjects in each group. Vertical bars denote standard error of mean. Statistical comparisons are done between NHS and other groups in both Basal MCP-1 expression (a) and stimulation with IL-15 and IL-12. *represent significant difference p<0.05

The increased chemokine expression in HIV infection, unlike HIV-TB, might suggest that TB influences the chemokine response in HIV-infected patients. A correlation study analyzing the expression of corresponding chemokine receptors for these chemokine ligands in the same subjects will aid in understanding the chemokine response of NK cells better.

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

Cytotoxic cell response in *M. tuberculosis* infection

Background

The proteins, early secreted antigenic target-6 (ESAT-6) and CFP-10 are reported as potent T-cell antigens. The region encoding these proteins is referred to as region of deletion-1 (RD1) which is present in *M. tuberculosis*, but not in *M. bovis* BCG. Although implicated in virulence, they also have a role in providing protection against TB. Screening minimal putative epitopes in these proteins may aid in developing a diagnostic test for TB or designing vaccines.

Aim

- To study the minimal putative epitopes present in ESAT-6 and CFP 10 to those HLA molecules which have been typed in our study

Methods

Major histocompatibilty complex (MHC) binding peptides (15-mers) were predicted from ESAT-6 and CFP-10 using MHC-II binding prediction server available at *http://tools.immuneepitope.org/main/jsp/menu.jsp*. For prediction, the consensus method was chosen, since the method has been reported to be better than other methods. The potential nonameric epitopes were predicted using Propred available at *http://www.imtech.res.in/raghava/propred*. Default threshold was set at which the sensitivity and specificity were found to be similar. Human leucocyte antigen (HLA) class II alleles were typed in our study only by low resolution (two digits), while in the bioinformatics server that we used, higher resolution for HLA were available. In both the methods, HLA were restricted by HLA subtypes observed in our study population.

Results

In the previous Annual report (2007-2008), IFN-γ and IL-4 responses to ESAT-6 and CFP-10 peptides (9 peptides each) in CD4 and CD8 cells were shown. In that report, we showed that ESAT-6_{51-70} gave an enhanced IFN-γ/IL-4 ratio in HHCs compared to PTB patients. But CFP-10 peptides did not elicit such an enhanced ratio. Bioinformatic analysis of both ESAT-6 and CFP-10 related to the HLA molecules typed in our study has now been done.

Potential epitopes present in the ESAT-6 and CFP-10 were predicted using bioinformatics methods. When ESAT-6 was studied, 15-mers which were found to have starting position in between 63-69, that is part of both Esp6 and Esp7 which also elicited *in vitro* immune response in our study. When Esp6 and Esp7 were analysed using ProPred, nonamer 69-77 was predicted as a potential promiscuous epitope as it was predicted to be binding with 30 different HLA subtypes (table 22).

Table 22: Potential epitopes in ESAT-6 preidcted by *in silico* methods

Sl. No	20-mers	*In vitro* response	Starting position of 15-mers	HLA predicted using IEDB	Start posi-tion of 9-mers	HLA predicted using ProPred
1	1-20		2, 3, 4, 5 and 6	HLA-DRB1*0101	6	DRB1_0305, DRB1_0401, DRB1_0426, DRB1_1114, DRB1_1323
			5	HLA-DRB1*0701	8	DRB1_0101, DRB1_0401, DRB1_0405, DRB1_0408, DRB1_0426, DRB1_0802, DRB1_1101, DRB1_1128, DRB1_1305, DRB1_1307
			12, 13, 14, 15, 16, 17, 18	HLA-DRB1*0404	18	DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0311, DRB1_0401, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0408, DRB1_0410, DRB1_0421, DRB1_0423, DRB1_0426, DRB1_1107, DRB1_1304
			17	HLA-DRB1*0405		
2	11-30	No	12, 13, 14, 15, 16, 17, 18	HLA-DRB1*0404	18	DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0311, DRB1_0401, DRB1_0402, DRB1_0404, DRB1_0405, DRB1_0408, DRB1_0410, DRB1_0421, DRB1_0423, DRB1_0426, DRB1_1107, DRB1_1304
			17	HLA-DRB1*0405	22	DRB1_0405, DRB1_0410, DRB1_1321
3	21-40	No	30,31,32	HLA-DRB1*0802	22	DRB1_0405, DRB1_0410, DRB1_1321
4	31-50		31,32	HLA-DRB1*0802	43	DRB1_0401, DRB1_0421, DRB1_0426, DRB1_0701, DRB1_0703
5	41-60	No			43	DRB1_0401, DRB1_0421, DRB1_0426, DRB1_0701, DRB1_0703
					51	DRB1_1321
6	51-70	Yes	63, 64, 65, 66	HLA-DRB1*0301	51	DRB1_1321
			66	HLA-DRB1*0401	69	DRB1_0301, DRB1_0305, DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0309, DRB1_0311, DRB1_0804, DRB1_0813, DRB1_1101, DRB1_1102, DRB1_1104,
						DRB1_1106, DRB1_1107, DRB1_1114, DRB1_1120, DRB1_1121, DRB1_1128, DRB1_1301, DRB1_1302, DRB1_1304, DRB1_1305, DRB1_1307, DRB1_1311
			63, 64, 65, 66, 67, 68	HLA-DRB1*1101		DRB1_1321, DRB1_1322, DRB1_1323, DRB1_1327, DRB1_1328, DRB1_1506
			63, 64, 65, 66, 67, 68, 69	HLA-DRB1*1302		
			63, 64, 65, 66	HLA-DRB1*1501		
7	61-80	Yes	63, 64, 65, 66	HLA-DRB1*0301	69	DRB1_0301, DRB1_0305, DRB1_0306, DRB1_0307, DRB1_0308, DRB1_0309, DRB1_0311, DRB1_0804, DRB1_0813, DRB1_1101, DRB1_1102, DRB1_1104,
						DRB1_1106, DRB1_1107, DRB1_1114, DRB1_1120, DRB1_1121, DRB1_1128, DRB1_1301, DRB1_1302, DRB1_1304, DRB1_1305, DRB1_1307, DRB1_1311
			66	HLA-DRB1*0401		DRB1_1321, DRB1_1322, DRB1_1323, DRB1_1327, DRB1_1328, DRB1_1506
			63, 64, 65, 66, 67, 68	HLA-DRB1*1101		
			63, 64, 65, 66, 67, 68, 69	HLA-DRB1*1302		
			63, 64, 65, 66	HLA-DRB1*1501		
8	71-90	Yes	-	-	-	-
9	79-95	No	-	-	-	-

The nonamers were predicted by Propred. HLA typing was carried out only at low resolution for HLA-DRB1*03, 04, 07, 10, 11, 12, 13, 14, 15 and 16.

Subtypes of these alleles were screened by *in silico* methods.

In the case of CFP-10 (data not shown), eight nonamers were found to be predicted as potential epitopes recognized by HLA-DRB1 subtypes. Among them, epitopes CFP-10_{56-64} and CFP-10_{76-84} were predicted as potential promiscuous epitopes as the former was predicted as potential binder with 32 different HLA-DRB1 alleles, while the latter was predicted to be recognized by 41 alleles. The remaining epitopes were predicted to be binders to HLA ranging from 1 to 8 different HLA-DRB1 subtypes.

In ESAT-6, only the first two nonamers, 6-14(WNFAGIEAA) and 8-16 (FAGIEAAAS) can be the possible epitopes of Esp1, which caused immune response *in vitro*. The Propred analysis showed that the nonamer 69-77, which was part of Esp6 and 7, would be a possible promiscuous epitope. For CFP-10, the minimal epitopes, CFP-10_{56-64} (VRFQEAANK) and CFP-10_{76-84} (IRQAGVQYS) were predicted as potential promiscuous nonamers by ProPred.

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

Role of interferon gamma assay for latent TB in HIV infection

Background

A major break through in TB diagnosis is the advent of interferon gamma (IFN-γ) release assay (IGRA) with *M. tuberculosis* specific antigens. The published reports have evidenced that IGRAs are highly sensitive and least influenced by BCG and other environmental mycobacteria. IGRA has been used for TB infection surveys and incorporated into the routine clinical practice for detection of latent TB infection (LTBI) in low endemic countries. However, the usefulness of IGRA in high endemic countries has not been established.

Aim

- To measure the IGRA positivity in healthy individuals

Methods

Quantiferon TB gold in-tube (QFT-G) kit was used to perform the test and results were interpreted as per manufacturer's instructions. The study subjects were recruited from villages, schools, colleges and offices in and around Chennai. Only healthy subjects with no known family contacts or history of TB were included in the study.

Results

A total of 228 subjects were recruited to the study. Of them, 81 subjects were below 15 yrs, and only 2 (2%) of them were positive for QFT-G (table 23). Ninety five subjects were in the age range of 16 to 35 yrs, and 32% of them were positive for QFT-G. The remaining 52 subjects were >35 yrs, and 70% of them were positive for QFT-G.

Table 23: Positivity of QFT-G in healthy subjects

Study subjects	Test positivity (%)
	QFT-G
Overall (N=246)	29
Pediatric (N=83)	2
2-9 yrs (N=46)	0
10-15 yrs (N=37)	5
Adults (N=163)	42
16-25 yrs (N=69)	25
26-40 yrs (N=49)	35
>40 yrs (N=45)	76

Conclusions

In high endemic countries, people become infected not only due to close contact with TB patients in the family, but also from the environment. It may not be possible to differentiate active and LTBI using IGRA in the exposed and possibly infected subjects. We found that in our settings, 98% of children below 15 yrs of age were negative for QFT-G and it can be presumed that their infection from environment is very minimal. Hence, QFT-G positivity in children (<15 yrs) with no history of TB contact, can be interpreted as active TB. We conclude that QFT-G can be used as a diagnostic tool for active TB in children. However, sensitivity of QFT-G has to be tested in children with TB, which is our ongoing work.

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Role of chemokines in tuberculous immunity

CC-Chemokine levels and its receptor expression on immune cells during pulmonary tuberculosis

Background

Chemokines and their receptors orchestrate leukocyte recruitment and confer immunity during *M. tuberculosis* infection. The systemic changes in these immune mediators and their synchronized interaction that regulate the cell trafficking determines the fate of infection.

Aims

- To evaluate the *ex-vivo* levels of cytokines and chemokines that are essential in anti-tuberculous immunity
- To dissect the relevant chemokine receptor expression on various immune cells of patients with PTB

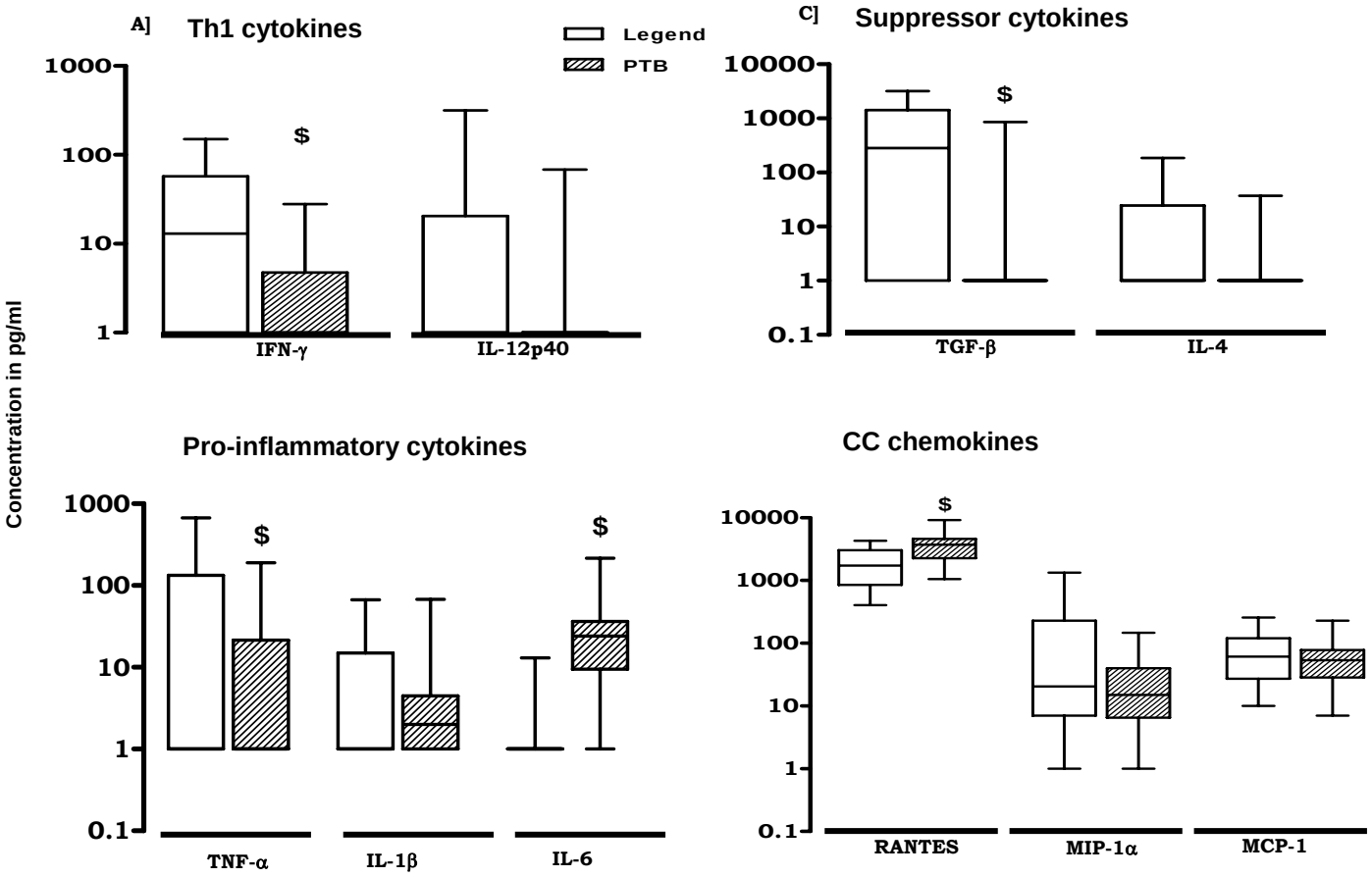
Methods

Cytokines and chemokines were evaluated by cytometric bead array and ELISA, and the expression of CC chemokine receptors was assessed by flowcytometry.

Results

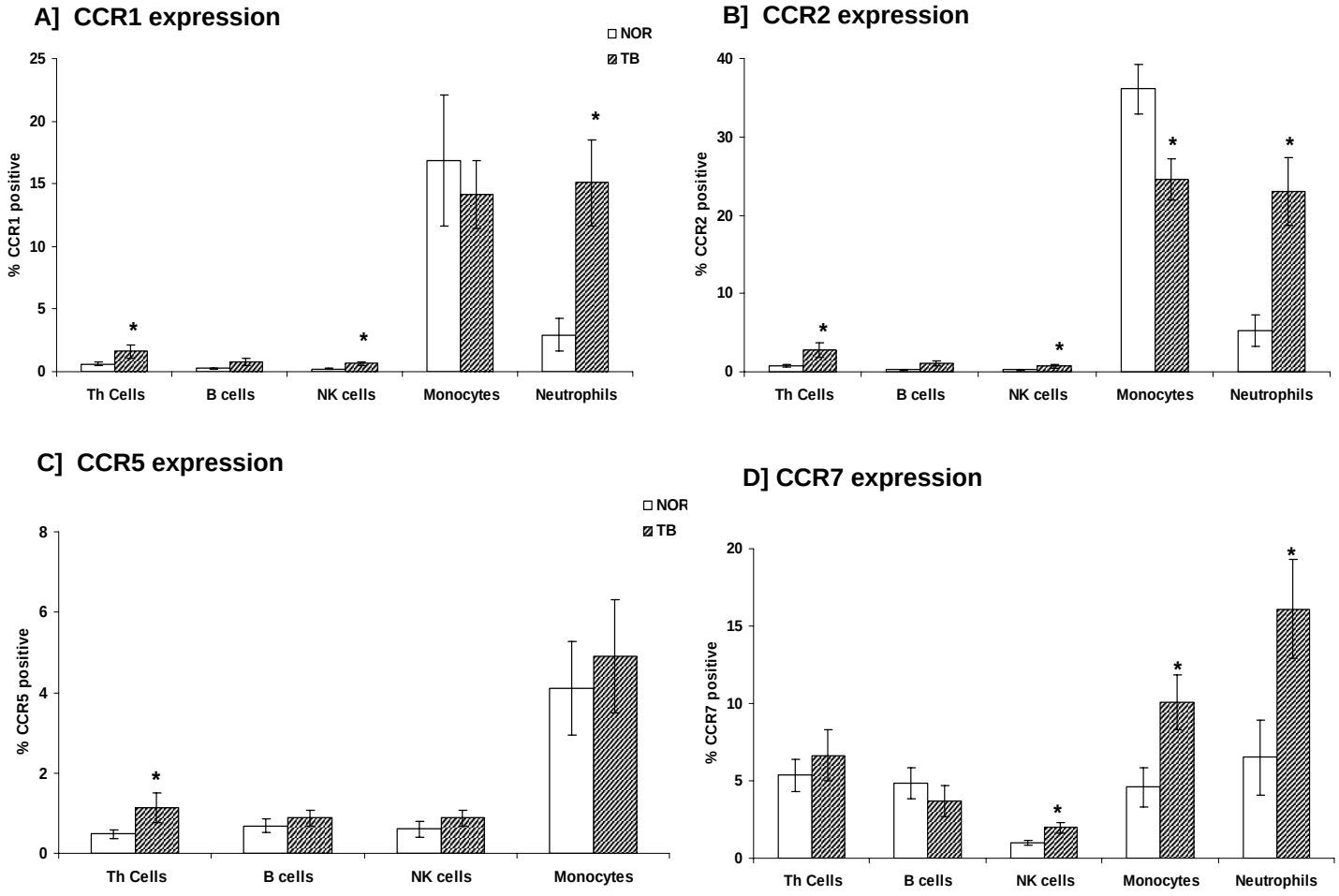
A significant decrease in IFN-γ, TNF-α and TGF-β and increase in RANTES and IL-6 was observed during PTB (Fig. 18). Significant correlation within chemokines and between cytokines was also observed in PTB. All immune cells except monocytes and B-cells significantly expressed higher levels of CCR1, CCR2, whereas CCR7 expression was up regulated only on monocytes and neutrophils in PTB (Fig. 19).

Fig. 18: Cytokine-chemokine response in PTB



$ $P<0.05$ compared with normal group

Fig. 19: CC-chemokine receptor expression on immune cells in PTB



*P <0.05 compared with normal group

Conclusion

Chemokines function coordinately and consistently during PTB. This balanced chemokine and cytokine relationship at the periphery may aid in amplified effector immune cell trafficking and retarded monocyte migration through differential chemokine receptor expression.

[Contact person: Dr. Sulochana D Das (E-mail ID: dsulochana@trcchennai.in)]

Role of dendritic cells in mycobacterial immunity - Evaluation of KG-1 cell line as an *in vitro* dendritic cell model for *M. tuberculosis* infection studies

Background

Dendritic cells play a fundamental role in initiating immunity during infection by controlling effector T-cell responses. Studies on DC in humans are difficult because of its low availability. Hence, a human cell line which can be easily propagated, maintained and differentiated into DC without much of cost and time to explore DC biology is the need of the hour.

Aim

- To evaluate KG-1, a leukemic cell line as an *in vitro* DC model for *M. tuberculosis* infection studies

Methods

KG-1 cell line was propagated in complete Iscove's modified Dulbecco's medium and allowed to differentiate in the presence of phorbol myristate acetate (PMA), ionomycin and granulocyte macrophage colony stimulating factor (GM-CSF) for different time periods (1, 3 and 5 days). The morphological changes were monitored using phase contrast and electron microscopy and phenotypic changes (MHC-II, CD 80 and CD 86) by flow cytometry. Endocytic capacity was measured by FITC-dextran uptake and T-cell stimulatory capacity was assessed through mixed leukocyte reaction.

Results

The combination of PMA + ionomycin + GM-CSF displayed the unique features of myeloid DC on fifth day. The expression of CD1a, MHC II, CD80, CD86 and CCR5 was significantly up regulated with increased uptake of dextran on fifth day

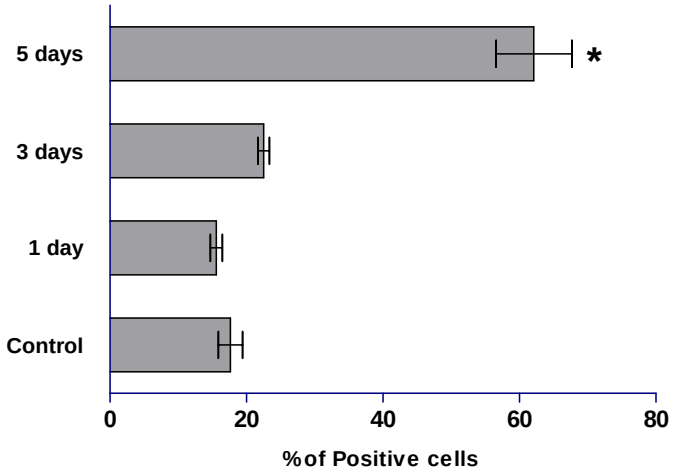
in differentiated KG-1 cells (table 24 and Fig. 20). Also fifth day observations differentiated KG-1 derived dendritic cells (KGDC) induced significant T-cell proliferation at all DC: T-cell ratios compared to controls (Fig. 21). After differentiation, KGDC acquired more apparent changes with long dendrite-like fine projections similar to stellate feature of DC with more granularities (Fig. 22).

Table 24: Dendritic cell marker expression on KGDC at different time points after treatment with PMA, ionomycin and GMCSF

CD markers (% positive cells)	Day 1	Day 3	Day 5
CD1a	0.4	6.4*	20.7*$
MHC II	69.3	76.9	86.8*$
CD80	2.5	15.6*	38.5*$
CD86	20.4	21.0	50.1*$
CCR5	2.3	5.4	25.3*$

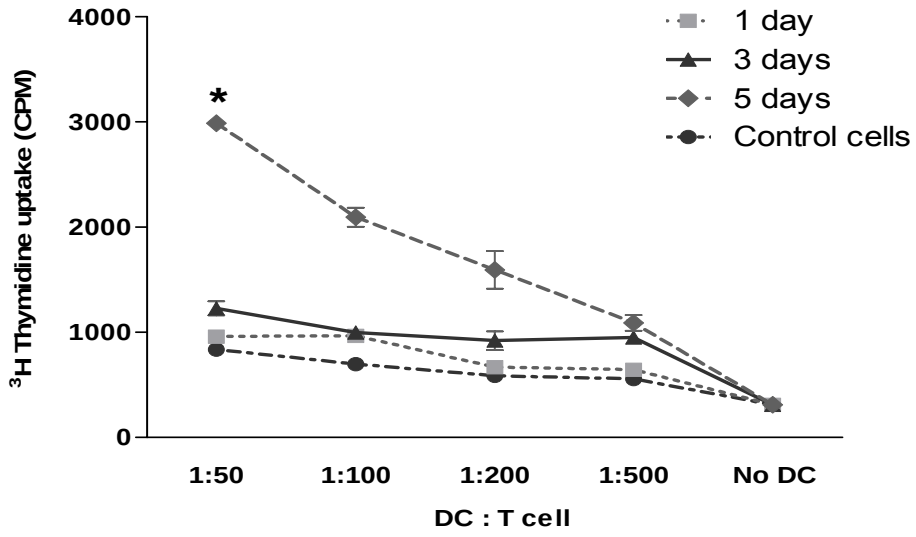
*compared with day 1 and $ compared with day 3 differentiated KGDC

Fig. 20: FITC dextran uptake by KGDC after differentiation at different time points



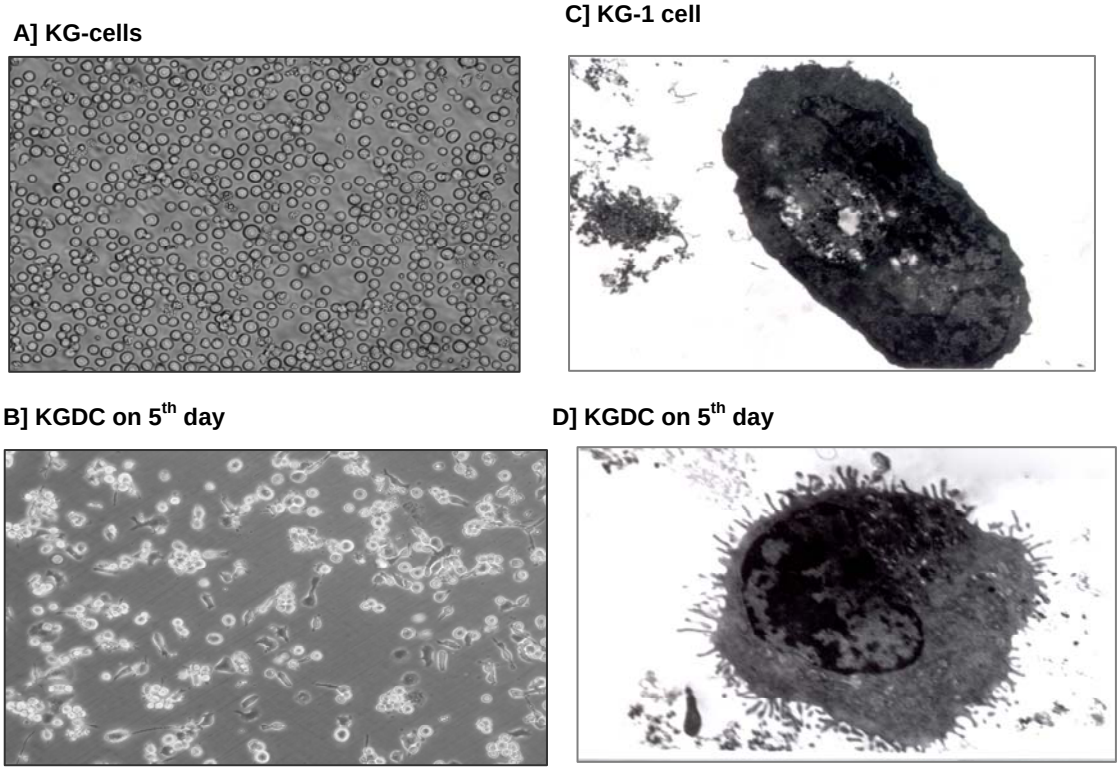
*compared with control KG-1 cells

Fig. 21: T-cell stimulating capacity of differentiated KGDC



*compared with control KG-1 cells

Fig. 22: Morphological changes in KGDC



The morphology of KG-1 cells is shown by phase contrast [A and B] and electron microscopy [C and D]. The morphological changes on 5th day are depicted in [B and D]

Conclusion

KG-1 cell line in the presence of PMA, ionomycin and GMCSF can be differentiated to DC morphology and phenotype.

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Molecular characterization of acetamidase operon of *M. smegmatis*

Background

Study of mycobacterial gene regulation at the promoter level is an important aspect of mycobacterial genetics. It is essential to understand the gene expression machineries of mycobacteria with relevance to transcription mechanisms, since many of the mycobacterial genes were not successfully expressed by the well established *E. coli* promoters. The highly inducible enzyme, acetamidase of *M. smegmatis* enables the organism to utilize several amide compounds as sole carbon source including acetamide and formamide. This is expressed at the basal level in non-induced conditions and gets 100 fold induced in the presence of an inducer such as acetamide. The acetamidase operon has four predicted open reading frames (ORFs), which are assumed to be involved in the regulation of this operon. Earlier we reported cloning of 4 ORFs (AmiC, A, D and AmiS) and purification of AmiA and AmiD and AmiA binding region in the operator region of acetamidase operon. *In vivo* over expression of AmiA in acetamide-induced cultures has shown negative regulation in amidase operon. We report further characterization of this regulatory protein by foot printing analysis, Electro mobility shift assay (EMSA) and protein-protein interaction studies.

Objective

- To characterize acetamidase operon of *M. smegmatis*

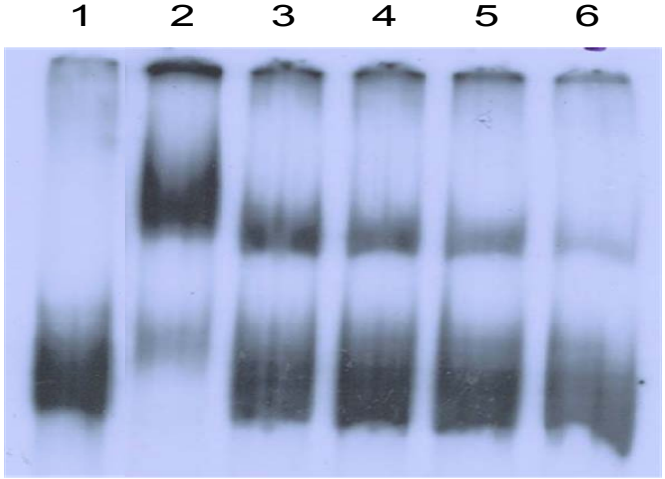
Results

EMSA showed that AmiA binds in the region (ML) between the *amiA* and *amiD* close to the predicted promoter. Cold chase assays showed that this binding is strong (Fig. 23). Foot printing analysis revealed that AmiA binds to a direct repeat sequence of GGGTGA spaced by eight bases (Fig. 24). DNA sequence

analysis showed that this direct repeat falls between the predicted transcription start site and ribosome binding site and two bases before the start codon of *amiD* gene. Foot printing results were further confirmed by cold chase experiments using double stranded oligos, where the specific palindromic sequence of the ML fragment to which AmiA was predicted to bind (Fig. 25).

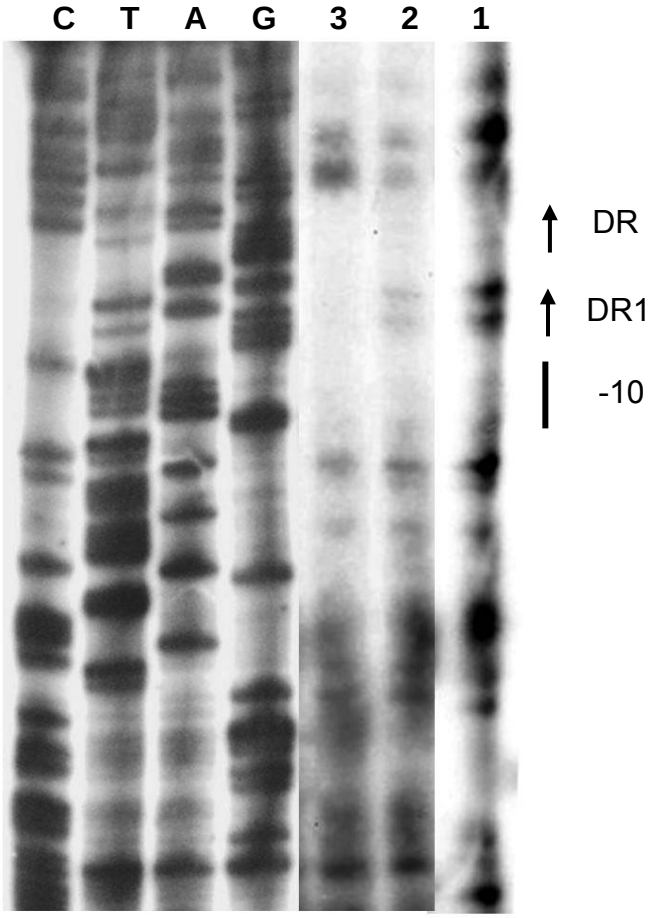
Protein–protein interaction of regulatory proteins present in the amidase operon was confirmed by Far western blotting. In this experiment we used purified AmiA (Fig. 26) and AmiD (Fig. 27) as bait proteins. The prey proteins, AmiA. AmiD and AmiC were over-expressed with hsp promoter in *M. smegmatis* mc^2155. AmiA and AmiD interacted with the lysate of these over-expressed recombinants. We found that an unknown common interacting prey protein was present in this lysate. This common interacting protein has molecular weight equivalent to that of AmiC. We also found that the interaction of these proteins occurred only in the presence of acetamide, and a similar binding was not observed in the absence of acetamide. From these results we hypothetically predict that the unknown protein could be AmiC. This will be further confirmed by protein sequence analysis.

Fig. 23: EMSA of AmiA with ML fragment



Lane 1: Free probe (FP). Lane 2: FP+30µg of AmiA.Lane 3: FP+ AmiA+10ng cold DNA Lane 4: 20ng cold DNA. Lane 5: 40ng cold DNA .Lane 6: 80ng cold DNA.

Fig. 24: Foot printing analysis



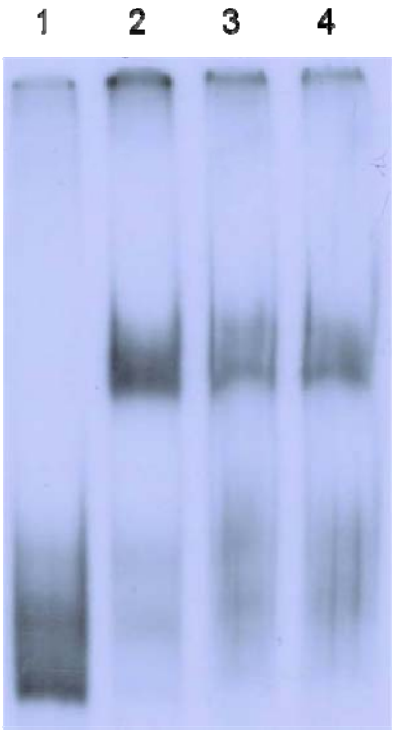
TSS RBS AmiD

GCGTTCACCC*TTGACT*TTTATTTTCATCTGGA*TATATT*TC<u>GGGTGA</u>ATGGAAAG<u>GGGTGA</u>CCATGCCGAC

'-35' '-10' DR1 DR2

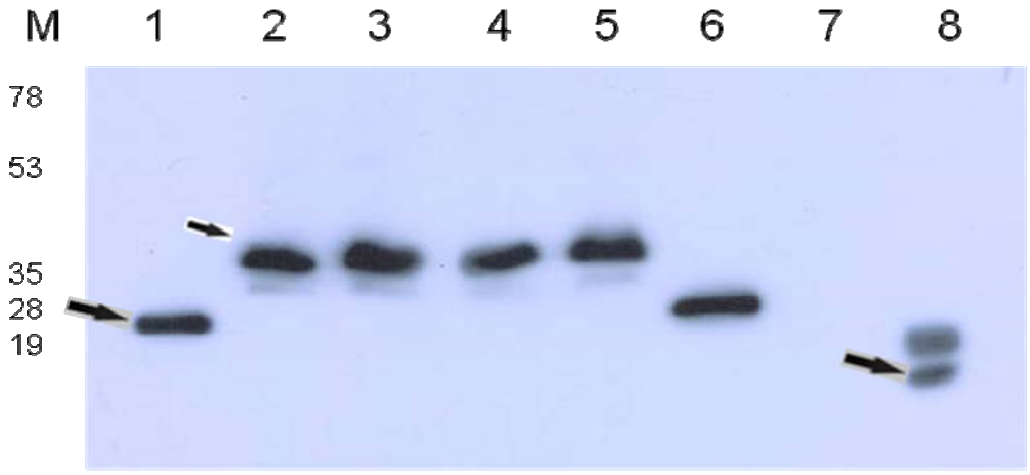
Foot printing of AmiA with ML fragment.Lanes G, A, T and C were loaded with the sequencing products of corresponding ddNTPs using USB PCR product sequencing kit with Sequenase enzyme. Lane 1 was loaded with foot printing reaction of ML probe with out specific protein using Promega Core foot printing kit with final DNase concentration of 0.3U/µl. Lanes 2 and 3 were loaded with foot printing reactions with 1nM and 2nM of AmiA protein.

Fig. 25: EMSA of AmiA with double stranded oligos



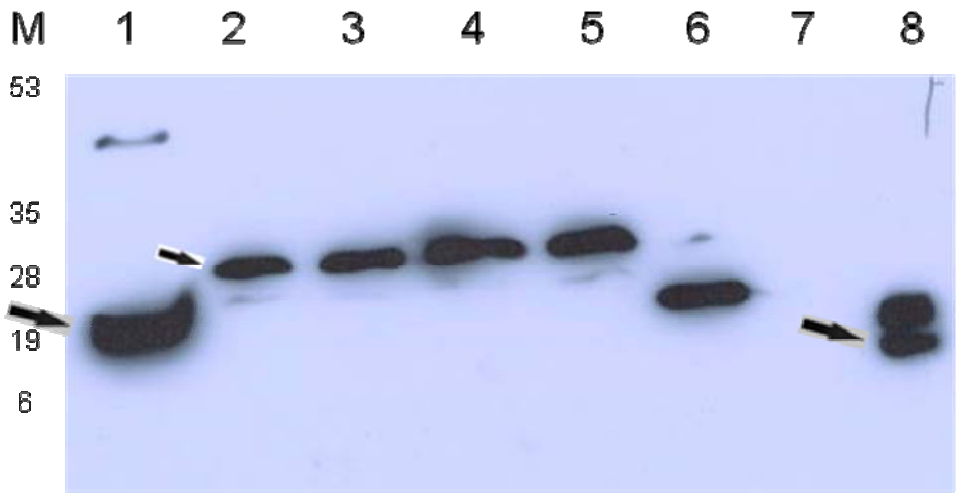
Lane 1: Free probe
Lane 2: ML + Protein
Lane 3: ML + Protein + Double stranded oligos (50 pmol)
Lane 4: ML + Protein + Double stranded oligos (100 pmol)

Fig. 26: Interaction of AmiA – Far western blotting



Lane 1: Purified AmiA
Lane 2: AmiA over expressed lysate
Lane 3: AmiD over expressed lysate
Lane 4: AmiC over expressed lysate
Lane 5: Mc^2 control lysate
Lane 6: AmiA Enterokinase cleaved
Lane 7: AmiD Enterokinase cleaved
Lane 8: Purified AmiD

Fig. 27: Interactions of AmiD – Far western blotting



Lane 1: Purified AmiA
Lane 2: AmiA over expressed lysate
Lane 3: AmiD over expressed lysate
Lane 4: AmiC over expressed lysate
Lane 5: Mc^2 control lysate
Lane 6: AmiA Enterokinase cleaved
Lane 7: AmiD Enterokinase cleaved
Lane 8: Purified AmiD

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PknE, a serine/threonine kinase from *M. tuberculosis* has a modulatory role in innate receptors during THP-1 human macrophage infection

Background

M. tuberculosis is a lung pathogen and resides within the alveolar macrophages. The bacteria have evolved strategies to evade the immune responses of the host, thereby leading a parasitic life in a persistant or dormant state. Eukaryotic-like serine/threonine protein kinases (STPKs) have become a lead family of study due to its regulatory role in signal transduction during pathogenesis. Our laboratory undertook a series of experiments to examine the role of PknE in host signaling. In our previous report, we have shown that the PknE deletion mutant modulates genes pertaining to apoptosis, immune response, metabolism and signal transduction.

Aim

- To study the effect of PknE deletion mutant on innate receptors (toll-like receptors) by using gene-disrupted and wild type (H37Rv) strains infected human macrophage-like THP-1 cells

Methods

THP-1, a human monocytic cell line was differentiated into adherent macrophages by the addition of PMA. The macrophages were infected with the strains H37Rv, ΔPknE. RNA was isolated from infected cells after 24 hrs post-infection. The quality of RNA was assessed by NanoDrop® ND-1000 spectrophotometer and Agilent 2100 bioanalyzer. The qRT-PCR based PCR array [Superarray Biosciences, USA] was used to validate the toll like receptors (TLR). The data were analyzed using software provided by the manufacturer.

Results

The microarray data of five days post infection showed differential regulation with respect to the deletion mutant in the TLR, and was chosen to be studied for a 24 hr period. The results indicated higher up-regulation of DNA processing and lipid detecting receptors (table 25).

Conclusion

Our data suggests for the first time a role for PknE in modulating the TLR receptor expression. The PknE might regulate the adaptive components that dictate the survival of bacilli inside the host. Further functional characterization of the serine/threonine kinase, PknE is being continued.

Table 25: Differential TLR gene expression at 24 hr post infection

S.No.	Gene name	Common name	Fold change H37Rv vs ΔPknE
1	CD14	CD14 molecule	0.648869383
2	TLR2	Toll-like receptor 2	2.291035796
3	TLR3	Toll-like receptor 3	3.620025852
4	TLR4	Toll-like receptor 4	2.542063379
5	TLR5	Toll-like receptor 5	0.164481816
6	TLR6	Toll-like receptor 6	5.449032139
7	TLR7	Toll-like receptor 7	2.259494429
8	TLR8	Toll-like receptor 8	6.993428963
9	TLR9	Toll-like receptor 9	4.550420911
10	TLR10	Toll-like receptor 10	4.979496942
11	CD180	CD180 molecule	6.088133523
12	LY86	Lymphocyte antigen 86	6.390815332

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STATISTICAL RESEARCH

PATTERN RECOGNITION METHODS FOR HIGH DIMENSIONAL DATA:

An application to FTIR spectral data

Background

Pattern recognition is a term which encompasses a wide range of techniques for classifying data. Given a collection of objects characterized by a set of measurements made on each object, the goal is to find and predict a property of the objects that is not directly measurable itself. Pattern recognition has a number of advantages for analyzing spectroscopic data. It provides an unbiased method of analysis, useful for both research and clinical applications. It provides ways of identifying features which differ between different classes of data together with methods for classifying unknown objects. Pattern recognition analysis is useful for both image processing and spectroscopy, but plays a different role in the two types of data. For image processing the main emphasis is on producing reproducible techniques which will assist the human analyst in interpretation of the image, whether it is to identify and typify structures or to quantify some property. With spectroscopy, the emphasis is more on discriminating between spectra from different classes of samples, and reducing the large numbers of spectral features in order to make the available information more accessible.

Fourier Transform Infrared (FTIR) Spectroscopic method has been used extensively to investigate biological samples—either by *in vivo* or *in vitro* analysis of tissue extracts—and their systemic effects. The common finding in most studies, independent of the nucleus and the experimental parameters used, has been that the intensities of almost all infrared are altered with respect to normal tissue. Many reports about patterns of spectral changes associated with for given disease types, cell types and disease states have been reported. With the onset of a disease, it is found that the relative content of bio-molecules changes, thereby producing a patho-physiological change in their functions. As blood serves as the primary metabolic transport system in the body, its composition is an excellent indicator with respect to the metabolic condition of the patient.

These biochemical changes of blood are particularly significant in the case of diseases such as diabetes mellitus. Using FTIR it has been demonstrated that glucose, cholesterol, albumin, total protein, triglycerides and urea can be assayed with dried serum.

Fourier – transform has been commonly used for spectroscopic analyses in the mid – infrared region due to following advantages: (1) the collection efficiency of photon fluxes is high because light from the light source or the sample with a wide area and a wide angle of radiation can be guided into the spectroscope efficiently; (2) the detection efficiency of signals is high because all the wave–lengths are detected simultaneously and (3) high resolution can be obtained because its wave number precision is high.

Aim

- To compare the multivariate based pattern classification methods for the discrimination of diabetic from normal serum using FTIR spectroscopic data

Methods

In a Fourier Transform spectrometer, a time domain plot is converted into a frequency domain spectrum. Complicated time domain spectra could be transformed into frequency domain spectrum, and the actual calculation of the Fourier transform of such systems is done by means of high-speed computers. The other commonly used methods are principal component analysis (PCA) and partial least square (PLS) analysis. In real samples, there are usually different sources of variation that make up the spectrum, such as the constituents in the sample matrix, inter constituents' interactions, instrumental variation such as detector noise, changing, of environments during sample collection that effect the baseline and absorbance, and differences in sample handing. These variations are presented in the collected spectral data at each wave length. The method used in the PCA statistical technique is that at characterized variations in the spectral data are determined, and these are used to construct the original spectrum by multiplying each one by a different constant scaling factor and adding the results factor. These variations are called principal components,

eigenvectors, spectral loadings or loading vectors and they are orthogonal to each other. The scaling constants used to reconstruct the spectra are known as scores and they are unique to each separate principal component. The first principal component accounts for the much of the variability in the data as possible, and each succeeding principal component accounts for as much of the remaining variability as possible. Reconstructed spectra data by PCA is obtained by using the goal of PCA to reduce the dimensionality of the spectra data and finally mean square sense is used to compare with original spectra data. The two step multivariate pattern recognition method of principal component regression is commonly used: in the first step, a Principal Component Analysis, PCA, of the data matrix X is performed. The measured variables (e.g., absorbance at different wavelengths) are converted into new ones (scores on latent variables). This is followed by a multiple linear regression step (MLR), between the scores obtained in the PCA step and the characteristic y to be modeled and MLR. PCA creates new orthogonal variables (latent variables) that are linear combinations of the original x-variables.

The PLS which includes indirect calibration modeling approach helps us to do multivariate calibration based on the least squares criterion. With respect to MLR, it has been traditionally used for the modeling of matrix Y by means of X. PLS possesses the distinct advantage of being more adaptable to modern measuring instrumentation, such as FTIR spectroscopy, which provide a large number of strongly correlated X-variables, also called predictors. In PLS projection method, the scores are linear combinations of the original variables Xk, and hence, these scores have the characteristic of weighed averages, being normally distributed and precise. Consequently, by selecting and combining the variables to few groups called scores, PLS may be useful to analysts to better interpret the large number of variables associated with the data. In PLS method the relationship between the predictors' variance and the dependent variables is represented by principal components that follow a numeric sequence, depending upon the strength of the relationship. Predictors' variables are considered significant when they take part in the creation of a principal component and,

consequently, all principal components are modeled based on the influence of each variable. A set of a number of principal components sufficient to give an exhaustive description of the Y-matrix is called model. If the model includes all the samples it is termed a calibration model. One of the advantages in using PLS method is that principal components are modeled not only on the predictors set, but also on the responses, so that it is possible to maximize the variance of both X and Y coordinates of the model. PLS is different from other multivariate calibrations, such as principal component regression, because the utilization of the responses data set is accomplished in an active way during the statistical calculations. By this way the information contained in X and Y coordinates are well balanced, and the effect of heavy but irrelevant variations in the predictors set is reduced.

Data

A state of high glucose level in the blood is recognized as diabetes. This state can be produced by different factors. Basically in diabetes there is a disturbance of metabolic function of all body cells and tissues. The cause for this metabolic disturbance relates to the deficiency of an anabolic protein hormone called “Insulin”, which is an internal secretion of the pancreas. Lack of insulin affects the metabolism of carbohydrate, fat and protein and it causes a significant disturbance of water and electrolyte homeostasis. The IR spectrum of serum can provide qualitative and quantitative information on such biomolecules. The data consisted of 11 normal and 18 diabetes (non insulin dependent diabetes mellitus), and FTIR data was measured in the wave length of 400 – 4,000. The spectral region consists of three regions, which corresponds to the glucose region (925-1250 cm-1); protein region (1500-1700cm-1) and lipids or fat region (2800-3400 cm-1). Considerable spectral differences observed between the normal and diseased serum were considered for application of pattern recognition.

Results

There were considerable variations between the patients and controls. Wave lengthwise comparison was made for the glucose region (1250 – 925 cm^{-1})

between the cases and controls. It was found that there was a significant difference in the FTIR valves between diabetic and controls in the regions 1250-1206 cm-1 and 1164-938 cm-1, whereas there was no significant difference in the other regions. The diabetic patients had consistently higher mean values compared to normals throughout the glucose region.

The principal component regression and PLS are the two most commonly used techniques in pattern recognition in high dimension databases. The principal component regression is a two step procedure; in the first step, PCA of the data matrix is performed, and the absorbance of the variables is measured at different wavelength and is computed in the latent variable. This is followed by a MLR between the scores in the PCA step and the characteristic of Y to be modeled. The PCA creates new orthogonal variables that are linear combination of the wavelengths which are highly correlated. The PLS is a generalization of MLR and PLS possesses the distinct advantage of being more acceptable to modern measuring instruments such as FTIR Spectroscopy which provides a large number of strongly correlated X-variables. In PLS projection the scores are linear combination of X's and weighted averages. One of the major advantage of PLS is that principal components are modeled not only on the predictors, but also on the responses, so that it is possible to minimize the variance of both X and Y co-ordinates of the model. PLS is different from other multivariate calibration models such as principal component regression, because the utilization of the responses data set is accomplished in an active way during the statistical calculations.

Total wavelengths in the glucose region considered were 325. The PCA and PLS were applied to the diabetic data. The variations explained by the first few components are given in table 26. It was observed that about 99% of the variation was captured by the first 3 components in the predictors.

Table 26: PLS Model-variations explained by the components

PC	Variation explained for predictor(s)		Variation explained for response(s)	
	%	Cumulative %	%	Cumulative %
1	79.744	79.744	70.980	70.980
2	17.988	97.732	19.165	90.145
3	1.448	99.180	2.580	92.724
4	0.358	99.538	3.091	95.815
5	0.071	99.609	2.112	97.927
6	0.295	99.905	0.333	98.260

The discriminant analysis was carried out using the first three components, and the Jackknifing classification resulted in 97% correct classification as shown in table 27. Further studies are under progress for comparing these approaches with Machine learning approaches such as artificial neural networks, support vector machines and genetic algorithms. The study is in progress.

Table 27: Jackknifed Classification matrix

	Predicted		
Observed	Normal	Diabetic	% correct
Normal	10	1	91%
Diabetic	0	18	100%
Total	10	19	97%

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ELECTRONIC DATA PROCESSING

The Electronic data processing (EDP) division provides computerized services to all the departments in the TRC. The EDP division continues to provide data management support, including data entry/verification to various studies undertaken at 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.

Data entry, information process and e-mailing are the key requirements for a research organization. Upgradation of servers and other IT equipments that were undertaken in the previous year was maintained during the year.

This division helps in providing audio-visual system for presentation of research materials during conferences, meetings and training programmes held at the centre.

Most 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.

Six Data entry operators, three Data processing assistants, one Network coordinator (ICER project) and one EDP-In charge are working in this division.

The quantum of documents of epidemiological, clinical, laboratory and program based studies entered and verified from April, 2008 to March, 2009 is shown below.

No. of documents entered: 1,84,137

No. of documents verified: 1,84,994

A total of 1,47,260 records were processed for the on-going third resurvey of disease survey conducted at Tiruvallur district. Eleven panchayats' pre-printed cards, and nine panchayats' person's alphabetical name-wise and household-wise lists were supplied for the third resurvey.

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

LIBRARY & INFORMATION SERVICES

The TRC library subscribes to approximately 1200 current journals and houses around 10,000 volumes of books and bound volumes of journals. Apart from these materials, the library collections include annual reports, audiovisuals, CDROM, gratis book materials, photographs, reprints, slides, theses, etc. An increasing amount of library resources are provided online.

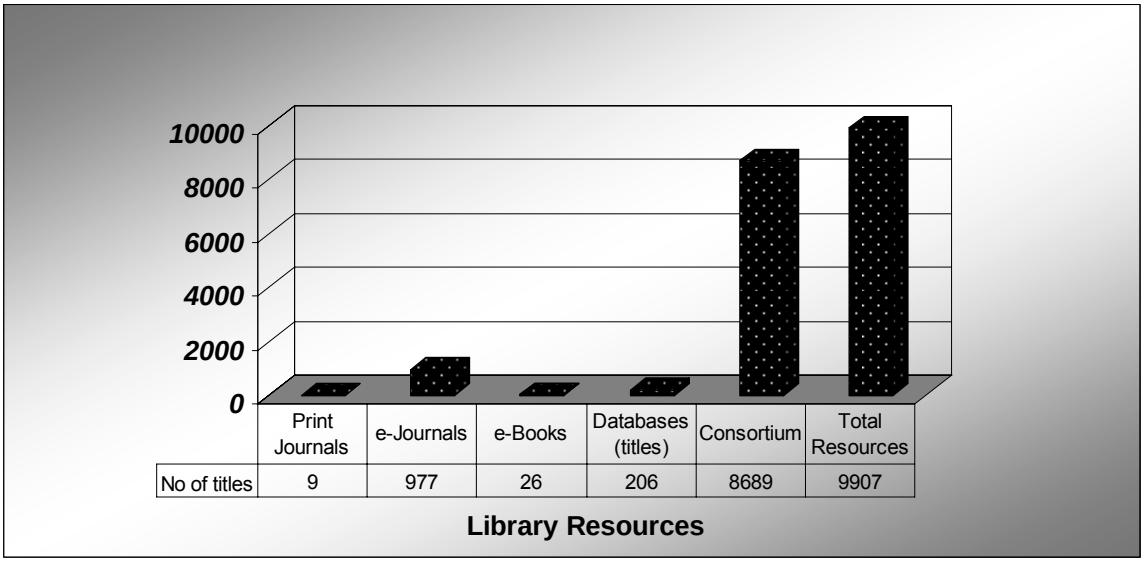
Library services

- Access to electronic resources (Digital library)
- Automation (borrowing & return)
- Catalogue (Online)
- Current awareness services (digital information alert - Intranet)
- E-mail coordination
- Internet browsing lab
- Inter library loan
- Reference assistance
- Resource sharing among ICMR libraries
- Using digital media resources
- Web based assistance

Gist of online collection

The TRC library provides online access to large number of e-journals, e-books, archives, cumulative titles, databases, subject collection and e-bundles. The collection building of library resources is illustrated in Fig.28. The scope of the library's collection is broad with excellent coverage on health sciences. Many of these have different structures and features. Digital library portal, a 24 hrs gateway, provides access to these resources.

Fig. 28: TRC library resources [Subscribed (Print/e-Journals/Databases); e-Books & Consortium]



Information about full-text access

The scientists/staff of the TRC can access the library's online resources via digital library through intranet/campus wide terminals connected with main server. Access to online full-text journals is restricted for remote access.

Consortium

In order to coordinate activities with other ICMR organizations for easier communication and networking, a consortium has been established for the following:

- Individual titles 5 Journals
    - BMJ; Lancet; Nature; NEJM; Science
- JCCC@ICMR (see Fig. 29) 1932 Journals
- J-Gate@ERMED (see Fig. 30) 862 Journals
    - OVID (2)
    - INFOTRAC Indian Medical Journal Collection (55)
- J-Gate 5890 Journals

Fig. 29: Screenshot of JCCC@ICMR

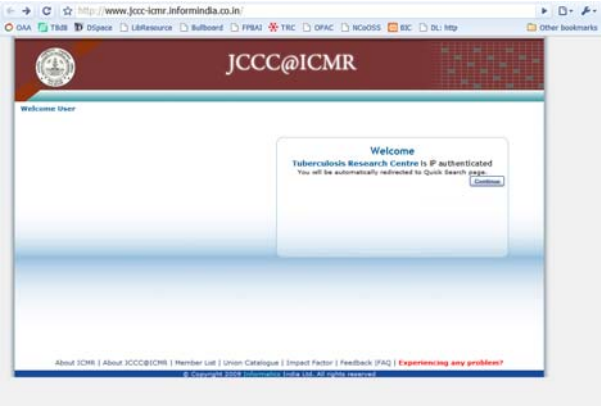


Fig. 30: Screenshot of J-Gate@ERMED

TRC library has been actively involved in opening its gateway to these resources through the digital library platform.

NEW ADDITION

The following e-book and archives have been added to the library collection:

e-Books

1. Harrison's Principles of Internal Medicine
2. e-Books@OVID : 25 titles

Archives

1. Nature Archives (1950 -1996) Vol. 165-384
2. Science Classic (July 1880-1996) Vol. 1-274

TB Alert: An in house publication is compiled at the library and circulated among ICMR institutes.

Membership

The TRC library has British Council Library institutional membership. The institutional membership cards are distributed among staff of TRC when required.

Web site

The TRC web site is being designed and maintained by the library.

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TRC – INSTITUTIONAL ETHICS COMMITTEE ACTIVITIES

Tuberculosis Research Centre Institutional Ethics Committee conducted five meetings (four scheduled and one unscheduled) during the year 2008. All the meetings satisfied the quorum requirements.

The Chair, the Member Secretary, and one member was present during all 5 meetings; out of the remaining seven members, two members were present for 4 meetings, three members were present for 3 meetings, one member could not be present for any of the meeting as she was not available within the country.

Ten new protocols and one re-submission were reviewed during the year 2008. Thirty two ongoing reviews and four expedited reviews (three protocols) were also conducted. Ten case study files were closed as the projects were completed and summary report submitted.

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

1. The 'Initial Review Submission Form' and the 'Ongoing Review Submission Form' were revised.
2. 'Continued Bioethics Education' discussions were held during the course of the meetings and via electronic mail; 'Informed consent: theory and reality' was discussed in one of the meetings.
3. As TRC is holding US Federal Wide Assurance, an annual report on possible research misconduct for the year 2007 was filed as 'nil' with the Office of Research Integrity, US Department of Health & Human Services.

INTERNATIONAL CENTRE OF EXCELLENCE IN RESEARCH (ICER)

During the initial five year period of the ICER (between 2003-2008), apart from renovating and equipping the laboratory building, a modern IT backbone was established that enabled a secure computer network with both high speed connections to the NIH's online resources and software for clinical and basic laboratory research. Videoconferencing was put into place that allowed sharing of research presentations and interactive training. Furthermore, redundant systems (from air handling to backup generators) and preventative maintenance for equipment and the infrastructure were built.

Because training was always an integral part of the ICER concept, there were a large number of on-the-ground training forums as well as short term training opportunities for collaborating members of the TRC staff. Training sessions were based on the perceived needs of the TRC staff and included courses in GCP and GLP, biostatistics, biosafety, good accounting practices, clinical trials design, antiretroviral therapy, and HIV care among others. Three to six month laboratory based collaborative research was undertaken by a number of staff and students at the TRC that included work on multicolor flow cytometry in HIV, pharmacokinetics, human genetics of extrapulmonary TB, proteomics of mycobacteria, pulmonary immunology, advanced clinical microbiology in HIV, and mycobacterial genetics. Shorter term training was made available to several members of the clinical research staff in the United States, South Africa and in Uganda.

Both basic and clinically-relevant, a great effort was placed on both establishing clinical research protocols and making inroads into the understanding of the pathogenesis of lymphatic filariasis, HIV, and TB.

Based on these activities and achievements, the ICER programme has been extended for a further period of 5 years (2008-2013). The Joint statement extending this agreement was signed by the Hon'ble Minister for Health and Welfare, Government of India and the US Secretary for Health on the sidelines of the World Health Assembly meeting.

APPENDICES

LIST OF PUBLICATIONS

Publications	**:**	**68**	
Publications in		i)	International Journals : 54
		ii)	National Journals : 14
Others - Books	:	i)	International : 1
		ii)	National : 6
Accepted for publication in		i)	International Journals: 37
		ii)	National Journals : 10

International:

1 Bakshi S, Ramachandran G, Ramesh K, Hemanthkumar AK, Anitha S, Padmapriyadarsini C, Narendran G, Menon PA, Rajasekaran S, Swaminathan S. Study of *ABCB1* polymorphism (C3435T) in HIV-1-infected individuals from South India. Br J Clin Pharmacol.2008;65:791-792.

2. Cobelens FG, Heldal E, Kimerling ME, Mitnick CD, Podewils LJ, Ramachandran R, Rieder HL, Weyer K, Zignol M; Working Group on MDR-TB of the Stop TB Partnership. Scaling up programmatic management of drug-resistant tuberculosis: A prioritized research agenda. PLoS Med.2008;5:1037-1042.

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5. Gosoniu GD, Ganapathy S, Kemp J, Auer C, Somma D, Karim F, Weiss MG. Gender and socio-cultural determinants of delay to diagnosis of TB in Bangladesh, India and Malawi. Int J Tuberc Lung Dis.2008;12:848-855.

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9. Kumar V, Prabakaran L, Gomathi S, Kriakov J, Dusthakeer A, Balaji S, Chan J, Jacobs WR Jr., Narayanan PR. Characterization of temperate phage Che12 and construction of a new tool for diagnosis of tuberculosis. Tuberculosis.2008;88:616-623.

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12. Muniyandi M, Ramachandran R. Socioeconomic inequalities of tuberculosis in India. Expert Opin Pharmacother.2008;9:1623-1628.

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14. Narayanan PR, Gopi PG, Subramani R, Santha T, Chandrasekaran V, Muniyandi M, Radhakrishnan S. DOTS reaches socially marginalized population in the community : A study from a rural area of south India. J Commun Dis.2008;40:199-204.

15. Pokkali S, Das SD, Logamurthy R. Expression of CXC and CC type of chemokines and its receptors in tuberculous and non-tuberculous effusions. Cytokine.2008;41:307-314.

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17. Raja A, Ranganathan UD, Bethunaickan R. Improved diagnosis of pulmonary tuberculosis by detection of antibodies against multiple *Mycobacterium tuberculosis* antigens. Diagn Microbiol Infect Dis.2008;60:361-368.

18. Rajashree P, Das SD. Infection with prevalent clinical strains of *Mycobacterium tuberculosis* leads to differential maturation of monocyte derived dendritic cells. Immunol Lett.2008;117:174-180.

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26. Ravanan R, Venkatesan P. Some new approaches for modeling incubation in HIV/AIDS Epidemic. Int J Computers, Mathemat Sci App. 2008;2:293-305.

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29. Selvaraj P, Vidyarani M, Alagarasu K, Prabhu Anand S, Narayanan PR. Regulatory role of promoter and 3' UTR variants of vitamin D receptor gene on cytokine response in pulmonary tuberculosis. J Clin Immunol.2008;28:306-313.

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54. Vidyarani M, Selvaraj P, Raghavan S, Narayanan PR. Regulatory role of 1, 25-dihydroxyvitamin D_3 and vitamin D receptor gene variants on intracellular granzyme A expression in pulmonary tuberculosis. Exp Mol Pathol.2009;86:69-73.

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4. Prabha C, Supriya P, Das SD, Sukumar B, Balaji S. Leptin response in patients with tuberculous pleuritis. Indian J Med Res.2008;128:721-727.

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6 Selvakumar N, Premkumar M, Kumar V, Gopi PG, Sivagamasundari S, Prabhakaran E, Narayanan PR. Phenol ammonium sulphate basic fuchsin staining of sputum in pot for the detection of acid-fast bacilli. Indian J Med Res.2008;128:194-197.

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Others – Books:

International:

1. Banu Rekha VV, Swaminathan S. Drugs used in tuberculosis and leprosy. Book published by Eronson, Elsevier, titled, “Side effects of drug annuals – Edition 30”.2008;357-363.

National:

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2. Venkatesan P, Srinivasan R. Bayesian models for HIV-AIDS in India. A spatial analysis. In: Applied Bayesian Statistical Analysis. Proceedings of NSABSA,2008;51-56.

3. Venkatesan P, Ponnuraja C. Bayesian separate and joint modeling for controlled clinical trial data using BUGS. In: Applied Bayesian Statistical Analysis. Proceedings of NSABSA,2008;132-138.

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ACCEPTED:

International:

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

2. Babu S, Bhat SQ, Pavan Kumar N, Lipira AB, Kumar S, Karthik C, Kumaraswami V, Nutman TB. Filarial lymphedema is characterized by antigen-specific Th1 and Th17 proinflammatory responses and a lack of regulatory T cells. PLoS Negl Trop Dis.

3. Babu S, Bhat SQ, Kumar NP, Jayantasri S, Rukmani S, Kumaran P, Gopi PG, Kolappan C, Kumaraswami V, Nutman TB. Human type 1 and 17 responses in latent tuberculosis are modulated by coincident filarial infection through cytotoxic T-lymphocyte antigen-4 and programmed death-1. J Infect Dis.

4. Babu S, Bhat SQ, Kumar NP, Anuradha R, Kumaran P, Gopi PG, Kolappan C, Kumaraswami V, Nutman TB. Attenuation of toll-like receptor expression and function in latent tuberculosis by coexistent filarial infection with restoration following antifilarial chemotherapy. PLoS Negl Trop Dis.

5. Babu S, Kumaraswami V, Nutman TB. Alternatively activated and immunoregulatory monocytes in human filarial infections. J Infect Dis.

6. Banu Rekha VV, Jaggarajamma K, Wares F, Chandrasekaran V, Swaminathan S. Contact screening and chemoprophylaxis in the Indian TB control programme : A situational analysis. Int J Tuberc Lung Dis.

7. Basirudeen S, Rajasekaran S, Swaminathan S, Venkatesan P, Paulkumaran P, Raja A. Role of Interferon gamma release assay in active TB diagnosis among HIV infected individuals. PLoS One.

8. Bhat J, Rao VG, Gopi PG, Yadav R, Selvakumar N, Tiwari B, Gadge V, Bhondeley MK, Wares F. Prevalence of pulmonary tuberculosis amongst the tribal population of Madhya Pradesh, central India. Int J Epidemiol.

9. Gopalaswamy R, Narayanan S, Chen B, Jacobs WR, Av-Gay Y. The serine/threonine protein kinase PknI controls the growth of *Mycobacterium tuberculosis* upon infection. FEMS Microbiol Lett.

10. Hemanth Kumar AK, Ramachandran G. Simple and rapid liquid chromatography method for determination of moxifloxacin in plasma. Journal of Chromatography B.

11. Kolappan C, Subramani R. Association between Biomass fuel and pulmonary tuberculosis: A nested case-control study. Thorax.

12. Kumar V, Raghavan R, Nagamiah S, Chauhan LS. External quality assessment of smear microscopy by the National Reference Laboratory in nine states of India. Int J Tuberc Lung Dis.

13. Lakshmi R, Ramesh K, Narayanan PR, Swaminathan S. Antiretroviral drug resistant mutations at baseline and at time of failure of antiretroviral therapy in HIV-1 co-infected TB patients. AIDS Res Hum Retroviruses.

14. Madhavan J, Mitra M, Mallikarjuna K, Pranav O, Srinivasan R, Nagpal A, Venkatesan P, Kumaramanickavel G. KIF14 and E2F3 mRNA expression in human retinoblastoma and its phenotype association. Molecular Vision.

15. Mimiaga MJ, Thomas B, Mayer KH, Reisner SL, Menon S, Swaminathan S, Periyasamy M, Safren SA. Alcohol use and HIV sexual risk among MSM in Chennai, India. Int J STD & AIDS.

16. Nyamathi A, Thomas B, Greengold B, Swaminathan S. Perceptions and health care needs of HIV-positive mothers in India. Prog Community Health Partnersh.

17. Padmapriyadarsini C, Pooranagangadevi N, Chandrasekaran K, Subramanyan S, Thiruvalluvan C, Bhavani PK, Swaminathan S. Prevalence of underweight, stunting and wasting among children infected with human immunodeficiency virus in south India. Int J Ped.

18. Pokkali P, Das SD, Selvaraj A. Differential up regulation of chemokine receptors on CD56+ NK-cells and their transmigration to the site of infection in tuberculous pleurisy. FEMS Immunol Med Microbiol.

19. Raghavan S, Selvaraj P, Swaminathan S, Alagarasu K, Narendran G, Narayanan PR. Haplotype analysis of HLA-A, -B antigens and –DRB1 alleles in south Indian HIV-1 infected patients with and without pulmonary tuberculosis. Int J Immunogenet.

20. Raghavan S, Selvaraj P, Swaminathan S, Narendran G. Association of HLA –A*1101 with resistance and B*4006 with susceptibility to HIV and HIV-TB: An *in silico* analysis of promiscuous T cell epitopes. AIDS Res Hum Retroviruses.

21. Ramachandran G, Ramesh K, Hemanth Kumar AK, Jagan I, Vasantha M, Padmapriyadarsini C, Narendran G, Rajasekaran S, Swaminathan S. Association of high T allele frequency of CYP2B6 G516T polymorphism among ethnic south Indian HIV-infected patients with elevated plasma efavirenz and nevirapine. J Antimicrob Chemother.

22. Ramachandran R, Nalini S, Chandrasekar V, Dave PV, Sanghvi AS, Wares F, Paramasivan CN, Narayanan PR, Sahu S, Parmar M, Chadha S, Dewan P, Chauhan LS. Surveillance of drug-resistant tuberculosis in the state of Gujarat, India. Int J Tuberc Lung Dis.

23. Ramana Rao PV Kumar MK, Basirudeen S, Sharma P, Soumya S, Raja A. Improved diagnosis of tuberculosis in HIV-TB patients using RD1 encoded antigen CFP-10. Int J Infect Dis.

24. Raparti V, Chitre T, Bothara K, Kumar V, Dangre S, Khachane C, Gore S, Deshmane B. Novel 4-(morpholin-4-yl)-N0-(arylidene) benzohydrazides: Synthesis, anti-mycobacterial activity and QSAR investigations. Eur J Med Chem.

25. Ravanan R, Venkatesan P. A simulation study on uncertainties associated with back calculation methodology to HIV/AIDS model. Int J Inf Sci Comp.

26. Sekar B, Arunagiri K, Selvakumar N, Serena Preethi K, Menaka K. Low frequency of moaA3 gene among the clinical isolates of *Mycobacterium tuberculosis* from Tamil Nadu and Pondicherry - south eastern coastal states of India. BMC Infect Dis.

27 Selvaraj P, Prabhu Anand S, Harishankar M, Alagarasu K. Plasma 1,25 dihydroxyvitamin D_3 level and expression of vitamin D receptor and Cathelicidin in pulmonary tuberculosis. J Clin Immunol.

28. Senbagavalli P, Geetha ST, Venkatesan P, Ramanathan VD. Defective solubilization of immune complexes and activation of the complement system in patients with pulmonary tuberculosis. J Clin Immunol.

29. Shenoy RK, Suma TK, Kumaraswami V, Rahmah N, Dhananjayan G, Padma S. Antifilarial drugs, in the doses employed in mass drug administrations by the Global programme to eliminate lymphatic filariasis, reverse lymphatic pathology in children with *Brugia malayi* infection. Ann Trop Med Parasitol.

30. Sulochana S, Mitchison DA, Kubendiren G, Venkatesan P, Paramasivan CN. Bactericidal activity of moxifloxacin on exponential and stationary phase cultures of *Mycobacterium tuberculosis.* J Chemother.

31. Thomas BE, Nyamathi A, Swaminathan S. Impact of HIV/AIDS on mothers in southern India: A qualitative study. AIDS and Behav.

32. Thomas BE, Mimiaga MJ, Mayer KH, Menon S, Chandrasekaran V, Murugesan P, Swaminathan S, Safren SA. HIV prevention programs in Chennai, India: are MSM being reached? AIDS Patient Care and STDs.

33. Thomas BE, Swaminathan S, Nyamathi A. Perceptions of health care needs of HIV positive mothers in India. Prog Community Health Partnersh.

34. Thomas BE, Mimiaga MJ, Menon S, Mayer KH, Chandrasekaran V, Murugesan P, Swaminathan S, Safren SA. Unseen and unheard: Predictors of sexual risk behavior and HIV infection among men who have sex with men in Chennai, India. AIDS Educ Prev.

35. Unissa AN, Selvakumar N, Hassan S. Analysis of pyrazinamide resistance in *Mycobacterium tuberculosis* by molecular modeling and docking. Bioinformation.

36. Venkatesan P, Suresh ML. Breast cancer survival prediction using artificial neural network. Int J Comp Sci Network Sec.

37. Wright A, Zignol M, Van Deun A, Falzon D, Gerdes SR, Feldman K, Hoffner S, Drobniewski F, Barrera L, van Soolingen D, Boulabhal F, Paramasivan CN, Kam KM, Mitarai S, Nunn P, Raviglione M; for the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Epidemiology of antituberculosis drug resistance 2002–07: an updated analysis of the global project on anti-tuberculosis drug resistance surveillance. The Lancet.

National

1. Alagarasu K, Selvaraj P, Swaminathan S, Raghavan S, Narendran G, Narayanan PR. CCR2, MCP-1, SDF-1α and DC-sign gene polymorphisms in south Indian HIV-1 infected patients with and without tuberculosis. Indian J Med Res.

2. Banu Rekha VV, Ramachandran R, Kuppu Rao KV, Rahman F, Adhilakshmi AR, Kalaiselvi D, Murugesan P, Sundaram V, Narayanan PR. Assessment of long term status of sputum positive pulmonary TB patients successfully treated with short course chemotherapy. Indian J Tuberc.

3. Hanna LE, Nayak K, Subramanyam S, Venkatesan P, Narayanan PR, Swaminathan S. Incomplete immunological recovery following anti-tuberculosis treatment in HIV-infected individuals with active tuberculosis. Indian J Med Res.

4. Hemanth Kumar AK, Ramachandran G, Rajasekaran S, C Padmapriyadarsini C, Narendran G, Anitha S, Subramanyam S, Kumaraswami V, Swaminathan S. Pharmacokinetics of lamivudine and stavudine in generic fixed-dose combinations in HIV-1 infected adults in India. Indian J Med Res.

5. Jaggarajamma K, Balambal R, Muniyandi M, Vasantha M, Thomas B, Nirupa C, Sudha G, Chandrasekaran V, Wares F. Perceptions of tuberculosis patients about private providers before and after implementation of Revised National Tuberculosis Control Programme. Indian J Tuberc.

6. Pokkali S, Rajavelu P, Sudhakar R, Das SD. Phenotypic modulation in *Mycobacterium tuberculosis* strains infected neutrophil during tuberculosis. Indian J Med Res.

7. Ramesh Kumar S, Swaminathan S, Flanigan T, Mayer KH, Niaura R. HIV & smoking in India. Indian J Med Res.

8. Supriya P, Priya R, Sudhakar R, Sulochana D. Phenotypic modulation in *Mycobacterium tuberculosis* infected neutrophil during tuberculosis. Indian J Med Res.

9. Thomas BE, Chandra S, Selvi KJA, Suriyanarayanan D, Swaminathan S. Gender differences in sexual behaviour among people living with HIV in Chennai, India. Indian J Med Res.

10. Thiruvalluvan E, Shenbagavalli R, Mohana M. Prevalence of HIV among tuberculosis out patient attendees. SAARC J Tuber Lung Dis HIV/AIDS.

Awards/Honours

- A patent related to "A process for the preparation of primers useful for the detection of *M. tuberculosis*" in the name of Indian Council of Medical Research has been granted patent number this year – **Dr. Sujatha Narayanan.**

- Tuberculosis Association of India – 1st Prize for Poster presentation entitled "POT Staining of sputum samples for the detection of AFB" during the NATCON 2007 held at New Delhi was awarded during September 2008 - **Dr.N. Selvakumar**.

Special Assignments

Dr.N. Selvakumar
Consultancy for the following:

- Laboratory Committee Meeting at N.T.I. Bangalore organized by Central TB Division, New Delhi, 8-9 May 2008
- Laboratory Committee Meeting at RNTCP, New Delhi, 5-6 June 2008.
- Procurement Committee Meeting organized by Central TB Division, New Delhi, 22 August 2008.
- Laboratory Committee meeting, New Delhi, 18 October 2008.
- National Task Force meeting, RNTCP, AIIMS, New Delhi, 22-23 October 2008.

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
- Training and guidance for four students (M.Sc.,) for their dissertation as a part of their syllabus (6 months)

Reviewer for journals:
International

- Clinical Chemistry and Laboratory Medicine
- International Immunopharmacology
- Expert Review of Anti-infective therapy
- BMC Infectious Diseases

National:

- Indian Journal of Medical Research
- Indian Journal of Tuberculosis

Reviewer of Project Proposals submitted to funding agencies:

- NHLS Research Trust, MRC-South Africa
- British Lung Foundation, UK

Student Advisory Committee:

- Guide for M. Phil., Dissertation of two candidates.

Project consultant:

- Prof. Lee Riley's laboratory, School of Public Health, University of California at Berkeley, USA for a period of 6 months from October 2008 - March 2009.

Dr. Sujatha Narayanan

- Examiner for M.Sc., Biotechnology examinations – Stella Maris College – April, 2008.

Dr.P.Selvaraj

- Executive council member, Indian society for Histocompatibility and Immunogenetics, New Delhi.

Reviewer for international journals:

♦ American Journal of Respiratory & Critical Care Medicine	-	Three
♦ Scandinavian Journa of Infectious Disease	-	One
♦ Human Immunology	-	Two
♦ International Journal of Tuberculosis & Lung Disease	-	One
♦ Infection, Genetics & Evolution	-	One
♦ Viral Immunology	-	One

External examiner for M.Sc. exams (theory & practicals):

- M.Sc., (Human Genetics) } Sri Ramachandra
- M.Sc., (Biotechnology) } University, Chennai.

Dr.D. Sulochana

- Doctoral committee member for two Ph.D. students from the Department of Zoology and Biochemistry, University of Madras, Chennai
- Reviewer for the Projects from DBT/DST funding agencies

Reviewer for International Journals:

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

Dr.P. Venkatesan

- Provided guidance to Project work for 4 M.Sc., (Biostatistics) and 5 M.Tech.,/M.Sc., Bioinformatics students from several colleges in and around Chennal
- Coordinated the works related to the Survey on “Evaluation of Kishori Shakti Yojana”
- Adjuvant Professor-Manipal University, Manipal
- Honorary Visiting Professor - Sri Ramachandra Medical University, Chennai
- Chairman - Institute Ethics Committee/Institute Review Board, Sri Ramachandra Medical University, Chennai
- Chairman - Board of Studies – M.Sc., (Bioinformatics) - Sri Ramachandra Medical University, Chennai
- Chairman - Board of Studies – B.Tech., (Medical Informatics) - Sri Ramachandra Medical University, Chennai
- Expert Member - Scientific Advisory Board, Sai’s Biosciences Research Institute, Chennai
- Member- Board of Studies – M.Sc., (Statistics) & M.Sc., (Biostatistics), University of Madras, Chennai
- Member – Board of Studies – M.Sc., (Statistics) & B.Sc., (Biostatistics), Manonmaniam Sundaranar University, Tirunelveli
- Member - Board of Studies – M.Sc., (Mathematics), Periyar University, Salem
- Member - Board of Studies – M.Sc., (Human Genetics) & B.Sc., (Biomedical Sciences), Sri Ramachandra Medical University, Chennai
- Member - Board of Studies – M.Tech., (Bioinformatics) and B.Tech., (Bioinformatics), Sathyabama University, Chennai
- Member – Board of Studies – M.Sc., (Mathematics), Meenakshi College for Women (Autonomous), Chennai
- External Examiner-University of Madras, Tamil Nadu Dr.MGR Medical University & Sri Ramachandra University, Chennai
- External Examiner – Ph.D. Viva-voce Examination, Dr. MGR University, Chennai
- External Examiner for evaluation of one Ph.D. thesis for the award of Ph.D. degree
- Nominated for question paper setting for graduate and post-graduate courses in University of Madras, The Tamil Nadu Dr MGR Medical University, Sri Ramachandra University, SRM University and Sathyabama University
- Member - Editorial Board: Journal of Pure and Applied Spectrophysics
- Joint Organizing Secretary – 2nd International Symposium on Global Trends in Biomedical Informatics, Research and Education, Chennai
- General Secretary - Indian Society for Medical Statistics
- General Secretary – International Biometric Society
- Reviewer of articles from four journals for their suitability for publication

Dr. Geetha Ramachandran

- Project guide for M.Phil., B.Tech. (Biotechnology) & M.Sc., students
- Reviewer of research proposals submitted to funding agencies.

Dr. Beena E. Thomas

- Faculty for NACO Specialist Training on “Confidentiality and treating minors’ on HIV Care and Treatment – organized by Govt. Hospital of Thoracic Medicine, Tambaram, Chennai.
- Panelist in the National Study Conference on “Social Work Profession – A Future Perspective” at the Department of Social Work, Loyola College, Chennai.
- External Examiner for the students of Post Graduate Students of Department of Social Work at Mar Gregorios College of Arts and Science, Chennai.

Conferences / Workshops / Training Programs Attended

1. Symposium on “Genes to Drugs: *In-silico* approaches” held at Pune during April 2008 – Jagadish Chandrabose & Sameer Hassan.

2. BioVision Alexandria 2008 Conference held at Alexandria, Egypt during April 2008 - Soumya Swaminathan.

3. Invited talk titled “Recent Advances in Computational Biology” presented at the National workshop on Mathematical aspects of scientific computing, held at Sathyabama University, Chennai during April 2008 – P. Venkatesan (Chairperson).

4. Ranbaxy Science Foundation – Challenges of MDR/XDR – Invited for panel discussion on reactivation/reinfection held at New Delhi during May 2008 - Sujatha Narayanan.

5. Invited talk at the ‘Brain storming session of Open source drug development - New drug development’ held at CSIR, New Delhi during May 2008 - Sujatha Narayanan.

6. Invited talk titled “Markov Chain Monte Carlo Methods in Bayesian Inference” presented at the UGC – National Seminar on Applied Bayesian Statistical Analysis held at Govt. Arts College, Salem, during May 2008 – P. Venkatesan.

7. Seminar on “Software reliability modeling using neural networks” held at the Centre for Reliability, Chennai, during May 2008 – P. Venkatesan.

8. Paper titled “Bayesian Separate and Joint modeling for controlled clinical trial data using BUGS” presented at the National Seminar on Applied Bayesian Statistical Analysis, held at Department of Statistics, Govt. Arts & Science College, Salem during May 2008 – C. Ponnuraja

9. Short Course in BioStatistics - Multiple Linear Regression, Logistic Regression in Survival Analysis held at Christian Medical College, Vellore during June 2008 - G. Narendran.

10. Workshop on Pediatric HIV held at Mumbai during June 2008 - Soumya Swaminathan.

11. Workshop on Clinical trials Registry India and Medical writing sponsored by NIMS, ICMR and WHO held at Bangalore during June 2008 – Banu Rekha VV.

12. Invited talk titled “Statistical methodological issues” presented at the National Statistical Day Seminar held at the University of Madras, Chennai, during June 2008 – P. Venkatesan.

13. Guest lecture on ‘‘Tuberculosis’ at the Respiratory Ailments Awareness Programme organized by the Nandalala Medical Foundation, Chennai, during July 2008 - M.S. Jawahar.

14. Workshop on “NIH Grants Policy and Management Training” held at New Delhi during July-August 2008 – Alamelu Raja.

15. Advanced Training in HIV/AIDS Clinical Trials held at Brown University, Providence, USA during July - November 2008 - Soumya Swaminathan.

16. Poster presentation (Psychosocial and demographic predictors of HIV risk and HIV infection in men who have sex with men in Chennai, India) at XVII International AIDS conference held at Mexico City, Mexico during August 2008 - Beena E. Thomas.

17. Zonal Task Force of Medical Colleges, South Zone, organized by Govt. Medical College, Thiruvananthapuram during August 2008–M.S. Jawahar.

18. Invited talk titled “Mathematical Models for Biomedical Applications” presented at the National Conference on Mathematical Modeling in Global Perspective at the Velammal Engineering College, Chennai during August 2008 – P. Venkatesan (Chief Guest).

19. Paper titled “How to write a scientific paper and critique of a scientific paper” presented at the Research Methodology Workshop held at SRM Medical College, Chennai, during September 2008 – P. Venkatesan (Resource person).

20. Workshop on “Data mining and Data Warehousing” held at Indian Statistical Institute, Kolkata during September 2008 – P. Venkatesan.

21. Paper titled “Statistical methods for clinical data management” presented at the Workshop on Informatics in Medicine held at Tuberculosis Research Centre, Chennai during September 2008 – P. Venkatesan (Resource person).

22. Paper titled ”Statistical methods for microarray data analysis” presented at the Symposium on Microarray Data Analysis held at SRM Dental College, Chennai during September 2008 - P. Venkatesan (Chief Guest).

23. NATCON–SEARO Conference 2008 held at New Delhi during September 2008:
Guest lectures titled: a) “Conducting Clinical Trials for TB treatment in India - Perspectives of the Principal Investigator”, and
b) “Shortening Tuberculosis Treatment – TRC experience”’-M.S. Jawahar.
c) “Emergence of XDR TB-TRC Experience” - Aleyamma Thomas.

24. Zonal Task Force (West Zone) organized by Goa Medical College, Goa during September 2008 - N. Selvakumar.

25. Guest lecture on “Pot method of staining Acid Fast Bacilli” at MICROCON 2008 held at Pune during September 2008 - N. Selvakumar.

26. Workshop on Biostatistics in Clinical Research held at New Delhi during September 2008 - Pradeep A Menon.

27. Meeting to approve the Memorandum of Understanding and Bye-laws of our society organized by the Association of MSSW Alumni held at Madras School of Social Work, Chennai during September 2008 - Dr. Beena E. Thomas.

28. Lecture on “Mycobacteriophage Genomics” held at Thanthai Hans Roever College, Perambalur, during September 2008 - Sameer Hassan.

29. Lecture on ‘Data Safety and Monitoring Boards’ during the Training Programme on Good Clinical Practices and Ethics, organized by the Indian Council of Medical Research under the Golden Triangle Partnership Scheme held at Chennai during October 2008 - M.S. Jawahar.

30. National Task Force (CME & Workshop) held at New Delhi during October 2008 - Aleyamma Thomas.

31. Paper titled “Impact of HIV/AIDS on Mothers in Southern India: A Qualitative Study” presented at NIMH International Research Conference on the Role of Families in Preventing and Adapting to HIV/AIDS, held at Providence, USA during October 2008 - Beena E. Thomas.

32. XXVI Annual National Conference of the Indian Society for Medical Statistics held at Nainital during October 2008:
a) “MCMC based calibration models for spectroscopic data” – P. Venkatesan.
b) “An empirical investigations from meta-analysis using randomized controlled clinical trials in a particular centre” - C. Ponnuraja.

33. Paper titled “A Phase 1 Study to Evaluate the Safety and Immunogenicity of a Recombinant Modified Vaccinia Ankara (rMVA) Virus Preventive Multigenic HIV subtype C Vaccine (TBC-M4) in Indian volunteers” presented at the Keystone Symposia ‘Pathogenesis & control of emerging infections & drug-resistant organisms’ held at Bangkok, Thailand during October 2008 - V.D. Ramanathan.

34. Paper titled “Multivariate Analysis – An Overview” presented at the Research Methodology Workshop held at PSG Medical College, Coimbatore, during October 2008 – P. Venkatesan (Resource person).

35. Invited talk titled “Multivariate analysis during SPSS” presented at the National Workshop on Applied Statistical Methods using SPSS held at Presidency College, Chennai, during October 2008 – P. Venkatesan (Resource person).

36. Invited talk titled “Machine learning approaches for pattern recognition” presented at the National Conference on Pattern Recognition held at Periyar University, Salem during October 2008 – P. Venkatesan.

37. Technical working group meeting on HIV-TB held at New Delhi (MOHFW/NACO) during November 2008 - Aleyamma Thomas.

38. Guest lecture on ‘Multi Drug Resistant Tuberculosis – the challenges ahead’ at the Regional CME organized by the Andhra Pradesh Chapter of the Association of Physicians of India and the Department of Medicine and Preventive Medicine of the Sree Venkateswara Medical College, Tirupati during November 2008 - M.S. Jawahar.

39. Guest lecture on “Advance in the development of Rapid Tests for the diagnosis of Pulmonary Tuberculosis” at NAPCON 2008 held at Lucknow during November 2008 – N. Selvakumar.

40. Guest lecture at 31st Andhra Pradesh TB & Chest Diseases Conference held at Nellore, Andhra Pradesh during November 2008 - Soumya Swaminathan.

41. Training on advanced genotyping techniques at Tufts University, Boston, USA during November 2008 - February 2009 – K. Ramesh.

42. Guest lecture on “Clinical features of opportunistic infections” in the CME on Pediatric HIV held at Puducherry during December 2008 - Soumya Swaminathan.

43. Invited talk on “Tuberculous pleuritis: A model to understand stress-induced Immunomodulation” at 4th DAE-BRNS Life Sciences Symposium (LSS-2008) on “Recent Advances in Immunomodulation in Stress and Cancer” held at Bhabha Atomic Research Centre, Mumbai during December 2008 – D. Sulochana.

44. Poster presentations at International Symposium on Emerging Trends in Tuberculosis Research - Biomarkers, Drugs & Vaccine held at ICGEB, New Delhi during December 2008 - D. Anbarasu, P.V. Ramana Rao, M. Madhan Kumar, S. Basirudeen & P. Rajashree.

45. Ranbaxy science foundation held at New Delhi during December 2008 - Aleyamma Thomas & Soumya Swaminathan.

46. 35th Annual conference of Indian Immunology Society held at Institute of Life Sciences, Bhubaneshwar during December 2008 - Alamelu Raja & P.V. Ramana Rao.

47. Workshop on “Monitoring progress towards millennium development goals for TB in India” held at Bangalore during December 2008 – C. Kolappan & R. Subramani.

48. Second SAARC Conference on TB & HIV/AIDS & Respiratory Diseases. held at Kathmandu, Nepal during December 2008 - N. Selvakumar, Prabu Seenivasan, V.N. Azger Dusthackeer, S. Balaji, M. Radhakrishnan, K Jaggarajamma & Mohanarani Suhadev.

49. Short course on Fundamentals of Biostatistics, Principles of Epidemiology, SAS (Statistical Analysis System) & Statistical Package for Social Science (SPSS), held at Christian Medical College, Vellore during December 2008 - Jagadish Chandrabose.

50. Guest lecture on ‘Funding Opportunities for Medical Research’ during the seminar in connection with the Platinum Jubilee celebrations of the Medical Council of India, organized by the Tamil Nadu Dr. MGR Medical University, Chennai during December 2008 - M.S. Jawahar.

51. IX National conference of FAIIE held at Tuberculosis Research Centre, Chennai during December 2008 – P. Venkatesan (Chair person).

52. Paper titled “Artificial neural networks” presented during the Refresher course for college teachers at Pondicherry University, Puducherry during December 2008 – P. Venkatesan (Resource person).

53. Workshop on “Bayesian Statistics using Open Bugs and R” held at St. Thomas College, Pala, during December 2008 – P. Venkatesan.

54. Annual endowment lecture at Govt. Rajaji Hospital, Madurai during January 2009 - Aleyamma Thomas.

55. Guest lecture at the Department of Biochemistry, Mohamed Sathak College, Chennai during January 2009 – Geetha Ramachandran.

56. Keystone on "Tuberculosis: Biology, Pathology and Therapy" held at Keystone, USA during January 2009 - Alamelu Raja.

57. Guest lecture on "INH preventive treatment for HIV-infected patients in India" at the 64th Annual Conference of Association of Physicians of India held at New Delhi during January 2009 – Soumya Swaminathan.

58. 61st Annual National Conference on Indian Psychiatric Society held at Institute of Mental Health & Hospital, Agra during January 2009 - Pradeep A. Menon.

59. Research dissemination workshop with Collaborators from Harvard Medical College, USA held at Tuberculosis Research Centre, Chennai during January 2009 - Beena E. Thomas.

60. Invited talk titled "Recent Advances in Biostatistical Methods" presented at the Technical Symposium on Biostatistical Tools and its Application held at SDNB Vaishnav College, Chennai during January 2009 – P. Venkatesan.

61. Paper titled "Stochastic Models for HIV/AIDS Projection" presented at the National Conference on Recent Advances in Pure and Applied Mathematics at DG Vaishnav College, Chennai during January 2009 – P. Venkatesan (Chief Guest).

62. Paper titled "Statistical Methods for Molecular Computing" presented at the Workshop on "Career oriented soft skills development in statistics" held at Bharathiar University, Coimbatore during January 2009 – P. Venkatesan (Resource person).

63. Guest lecture on 'TB epidemiology, control and drug resistance' at the Third International Short Course in Clinical Tropical Medicine, organized by Christian Medical College, Vellore during February 2009 - M.S. Jawahar.

64. Guest Lecture on “Recent advances in rapid diagnosis of MDR Tuberculosis” at the “Recent scenario of Co-infections in HIV/AIDS” sponsored by Tamilnadu State AIDS Control Society held at Institute of Microbiology, Madras Medical College & Government General Hospital, Chennai during February 2009 - N. Selvakumar.

65. Seventh International CALIBER 2009 - E Content Management: Challenges and Strategies”, held at Pondicherry University, Puducherry during February 2009 – R. Rathinasabapati.

66. Papers presented at the XI Annual Conference of Society of Statistics, Computer and Applications, held at Department of Statistics, University of Madras, Chennai during February 2009 - C. Ponnuraja & M. Vasantha.

67. Guest lecture at the National Seminar on Enzyme Technology at GR Damodharan College of Science, Coimbatore, during February 2009 – Geetha Ramachandran.

68. ICMR-Workshop on Clinical Data Management held at Mumbai during February 2009 - Jagadish Chandrabose & Sameer Hassan.

69. Oral presentation titled “Once daily Nevirapine versus Efavirenz in the treatment of HIV-infected patients with tuberculosis: A Randomized Clinical Trial” and poster presentations titled “Acquired Rifampicin Resistance in HIV-infected and –uninfected patients with TB treated with a thrice-weekly short-course regimen ” and “Innate and adaptive immunity in treatment-naïve HIV-infected pediatric patients in Chennai, India” at the 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009) held at Montreal, Canada during February 2009 - Soumya Swaminathan.

70. Invited talk on ‘Molecular epidemiology of *M. tuberculosis’* at the International symposium on Tribal Health held at Jabalpur during February 2009 - Sujatha Narayanan.

71. Workshop for sensitization of media regarding tuberculosis by NGO-REACH held at Chennai during February 2009 - Beena E. Thomas.

72. XI Annual Conference of the Society of Statistics, Computer and Applications held at University of Madras, Chennai during February 2009 – P. Venkatesan.

73. Paper titled “Bioinformatics tools and applications” presented at the National Conference on Recent Trends in Biotechnology held at Kamaraj Engineering College, Virudhunagar during February 2009 – P. Venkatesan.

74. Paper titled “Recent Advances in Applied Mathematics” presented at the Annual Mathematical and Statistical Association of JBAS College held at Chennai February 2009 – P. Venkatesan (Chief Guest).

75. Training of Trainers (Nurses) for DOTS plus held at LRS Institute of TB & Respiratory Diseases, New Delhi during March 2009 - Aleyamma Thomas.

76. Guest lecture on ‘Interventional Studies in Operational Research – Indications, merits and demerits’ at the RNTCP Operational Research Workshop, organized by Central TB Division, and NTI, Bangalore, during March 2009 - M.S. Jawahar.

77. Guest lecture on “Multi Drug Resistance in Tuberculosis – the Challenges ahead” on World TB Day, organized by SRM Medical College, Chennai, during March 2009 - M.S. Jawahar.

78. Glacial overview of tuberculosis held at St. James Pharmaceutical College, Chalakkudi, Kerala, during March 2009 – N. Selvakumar (Chair person), Vanaja Kumar, Gomathi Sekar, Prabu Seenivasan, Gomathi N.S., V.N. Azger Dusthackeer, S. Balaji, Sameer Hassan, R. Lakshmi & M. Radhakrishnan.

79. Workshop for TB Laboratory Technicians held at Male, Maldives during March 2009 - N. Selvakumar.

80. Guest lecture on “Managing urban crisis – Infectious diseases: low and high resource cities compare their plans to combat infection” at the BioVision 2009 World Life Sciences Forum held at Lyon, France during March 2009 - Soumya Swaminathan.

81. Consultants meeting on “Nutrition and Malaria, TB and other infectious diseases in infants and children” held at Vienna, Austria during March 2009 - Soumya Swaminathan.

82. LES CENT GARDES – “Latest Approaches to HIV Infection Management: A focus on HIV/TB and HIV/hepatitis co-infections” held at New Delhi during March 2009 – P.K. Bhavani.

83. Field Work Agency Supervisors’ meet held at The Madras School of Social Work, Chennai during March 2009 - Beena E. Thomas.

84. Invited talk titled (i) “Statistical Methods for Clinical Trials and (ii) Computation Molecular Biology Methods” at the Workshop on “Mathematical Statistics with Application” held at SDNB Vaishnav College for Women, Chennai during March 2009 – P. Venkatesan (Resource person).

85. Invited talk titled “Mathematical Modeling” at the Annual Mathematical Association Conference held at Savitha Engineering College, Chennai during March 2009 – P. Venkatesan (Chief Guest).

86. Paper titled “Artificial intelligence and Data Mining in Biomedical Informatics” presented at the 2nd International Conference on Biomedical Informatics and Signal Processing held at SSN College, Chennai during March 2009 – P. Venkatesan (Chairperson for 3 scientific sessions).

Workshops Organized by the Bio Informatics Centre

- **“**Perspectives of Biomedical Informatics conducted on June 02, 2008
- “Informatics in Medicine” conducted on September 22, 2008
- “Computational Resources for Drug Discovery” during November 24-26, 2008.

Advocacy:

- General Orientation Course for NSS programme officers of colleges in Tamil Nadu & Puducherry – Social workers division.

- Field work placement training for Medical Social work students from various city colleges – Social workers division.

- TB and HIV Awareness programme in various communities – Social workers division.

- A staff of Schizophrenia Research Foundation, Chennai, was given training on human DNA isolation and PCR technique from May 5 – 16, 2008 - Dr.P. Selvaraj.

- Training provided to B.Tech and Post graduate students from Biotechnology, Biochemistry and Molecular Biology disciplines - Dr.D. Sulochana

- One day training provided to M.Sc., Biochemistry students of G R Damodharan College of Arts & Science, Coimbatore – Dr. Geetha Ramachandran & Dr.A.K. Hemanth Kumar.

Ph.D. Scholars

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

Sl. No.	Name of the candidates	Title of the Ph.D. thesis	Supervisor/Guide
1.	Dr.G. Shenbagavalli	Serum and tissue complement profile in TB	Dr.V.D. Ramanathan
2.	Dr.V. Narayana Rao	Interaction of gene-disrupted *M. tuberculosis* strains with human complement system	Dr.V.D. Ramanathan
3.	Dr.M. Gomathi	Studies on sputum microscopy for detection of acid fast bacilli	Dr.N. Selvakumar
4.	Dr.M. Vidya Rani	Regulatory role of variant genotypes of Vitamin D receptor gene on Vitamin D_3 modulated cytokine and Granzyme-A response in pulmonary TB	Dr.P. Selvaraj
5.	Dr.R. Priya	Human monocyte and macrophage apoptosis induced by *M. tuberculosis* strains and its implication on cell mediated immune response	Dr.D. Sulochana
6.	Dr.P. Supriya	Chemokines and their cognate receptors in tuberculous immunity with special focus on plerual mesothelial cells	Dr.D. Sulochana
7.	Dr. Nisha Rajeswari	Influence of HLA-DRB1 alleles on macrophage phagocytosis, cytokine response and perforin positive cells in pulmonary TB	Dr.P. Selvaraj
8.	Dr.S. Manivannan	The role of complement activation and antibody in the interaction between *M. tuberculosis* and human mononuclear cells	Dr.V.D. Ramanathan

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

Sl.No.	Name of the candidate	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr.K. Alagarasu	Studies on Mannose binding lectin, CD209 and Vitamin D receptor gene polymorphisms in south Indian HIV-1 infected patients with and without TB	Dr.P. Selvaraj
2.	Ms.N.S. Gomathi	Rapid diagnosis and drug susceptibility testing of *M. tuberculosis*	Dr. Vanaja Kumar
3.	Dr.P.L. Natarajan	Cellular immunology of TB and HIV/TB	Dr. Sujatha Narayanan
4.	Mr. Kaustuv Nayak	Evaluation of cellular immune response to infection with HIV-1 C subtype in south India	Dr.P.R. Narayanan
5.	Mr.C. Ponnuraja	Frailty models	Dr.P. Venkatesan
5.	Ms. Mohanarani Suhadev	Sociological aspects of HIV/AIDS	Dr. Udaya Mahadevan
6.	Ms. Harini Laxminarayan	Study on molecular biology of *M. tuberculosis*	Dr. Sujatha Narayanan

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

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. thesis	Supervisor/Guide
1.	Mr.D. Anbarasu	CSIR	Identification & characterization of immunoreactive T-cell antigens of *M. tuberculosis*	Dr. Alamelu Raja
2.	Mr.P.V. Ramana Rao	ICMR	Innate immunity in HIV infection	Dr. Alamelu Raja
3.	Mr.M. Madhan Kumar	CSIR	Cytotoxic cellular response in TB	Dr. Alamelu Raja
4.	Mr.S. Basirudeen	ICMR	Interferon gamma assay for latent TB infection in HIV patients	Dr. Alamelu Raja
5.	Ms.S. Lakshmi	ICMR	HIV drug resistance	Dr.P.R. Narayanan
6.	Ms. Nusrath Unissa	ICMR	Molecular studies on isoniazid resistance in *M. tuberculosis*	Dr.N. Selvakumar
7.	Mr.S. Raghavan	ICMR	Human Leucocyte Antigen polymorphism studies in HIV and HIV-TB patients	Dr.P. Selvaraj
8.	Mr.S. Prabhu Anand	CSIR	Regulatory effects of vitamin D_3 & vitamin D receptor genotypes on VDR expression & cytokine production in PTB	Dr.P. Selvaraj
9.	Ms. Aparna J Christy	ICMR	Development of epitope delivery system for construction of recombinant BCG vaccine for TB	Dr. Sujatha Narayanan
10.	Ms.V. Malini	ICMR	Functional characterization of FtsY, a signal recognition particle receptor from *M. tuberculosis*	Dr. Sujatha Narayanan
11.	Ms.N. Yamuna	UGC	Classification and regression trees	Dr.P. Venkatesan
12.	Ms. Neema Bourai	CSIR	Functional characterization of serine/threonine protein kinase of *M. tuberculosis*	Dr. Sujatha Narayanan
13.	Mr.P. Dinesh Kumar	ICMR	A molecular approach to pathogenesis role of serine/ threonine kinase PknE in signal transduction involved in host pathogen interactions	Dr. Sujatha Narayanan
14.	Ms.P. Rajashree	ICMR	Role of dendritic cells in tuberculous immunity	Dr. Sulochana Das
15.	Mr.S. Balaji	ICMR	Rapid diagnosis of *M. tuberculosis*	Dr. Vanaja Kumar

Sl.No.	Name of the Candidate	Source of Funding	Title of the Ph.D. project	Supervisor/Guide
16.	Mr.M. Radhakrishnan	DST	Anti-TB drugs from actinomycetes	Dr. Vanaja Kumar
17.	Ms.R. Lakshmi	ICMR	Molecular studies on mycobacteria	Dr. Vanaja Kumar
18.	Mr. Sameer Hassan	ICMR-Biomedical Informatics Centre	Genome analysis of phages and viruses	Dr. Vanaja Kumar
19.	Mr. Jagadish Chandra Bose	ICMR-Biomedical Informatics Centre	Immunodominant epitopes against HIV subtype C	Dr. Luke Elizabeth Hanna

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

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

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