# TUBERCULOSIS RESEARCH CENTRE

CHETPUT CHENNAI – 600 031

## REPORT ON RESEARCH ACTIVITIES DURING 1998

INDIAN COUNCIL OF MEDICAL RESEARCH

NEW DELHI

# TUBERCULOSIS RESEARCH CENTRE

CHETPUT CHENNAI-600031


- TUBERCULOSIS RESEARCH CENTRE,
  Mayor V. R. Ramanathan Road,
  Chetput, Chennai - 600031, INDIA.
- **Telephone:** 826 5425 I 2 7 / 3 5 I 5 7
- **Facsimile:** +91 - (044) - 8262137
- **e-mail:** trcicmr@vsnl.com
- **Website:** http://www.trc-chennai.org


## Head Quarters


- INDIAN COUNCIL OF MEDICAL RESEARCH,
  (Ministry of Health & Family Welfare, Govt. of India)
  Post Box No. 491 1, Ansari Nagar,
  New Delhi-110029, INDIA.
- **Telephone:** 6962794/3980/2895
- **Facsimile:** +91 - (011) – 6868662
- **e-mail:** icrnrhqds@sansad.nic.in
- **Website:** http://WWW.icmr.nic.in

# PREFACE

Tuberculosis continues to be a major problem in our country. The increase in HIV infection and multidrug resistant *M.tuberculosis* is adding to the existing burden. The presently available tools if utilised properly would help a great deal in controlling the disease. However that has not happened for more than one reason. The nation is now at a stage where it is trying to harness the existing tools with a revised strategy in the most cost effective manner. The failure to control this dreadful disease in the past has highlighted the present necessity for more basic, applied and operational research to develop better tools and strategies for disease control.

The above statements are not mere repetition in the report of 1996 - 1997. They are still valid and this is a matter of concern. On behalf of my colleagues and on my own behalf, I am presenting the Annual Report of research activities that were carried out in 1998. I would like to share with you the direction in which the Tuberculosis Research Centre (TRC) is moving and the highlights of the last year's performance.

TRC is entering into a new phase of transformation as Mega Research Centre with a global view of tuberculosis research. Since 1997 we have been developing our new research strategy. During this period we have redirected our emphasis on operationalisation – together our strategy and resources to work in the control of TB. Our success now would be the basis for long-term commitment and leadership in tuberculosis research.

Traditionally the activities of the TRC were in three separate compartments, namely, clinical, epidemiological and basic research. We felt that these research activities would yield better results if they were managed in an integrated fashion. In order to achieve this objective we initiated a major project to establish a Model DOTS Centre that will function as a centre of excellence. This project would allow all aspects of research on tuberculosis to be conducted in a cohesive manner.

The Centre's new agenda will be to use the existing tools in a better manner in TB control, develop superior and newer tools and gain new knowledge. This Centre will create a network of people and systems, and develop alliances with national and international agencies that are involved in TB research and control. This Centre will also develop capabilities to act quickly and decisively and foster openness and initiatives to control TB.

In order to achieve these goals many human resource initiatives were undertaken within the organisation to complement the changes that are

envisaged in our newer strategy. It is obvious that human resource is a key element for any activity and keeping this in mind the training of many scientific, technical and administrative staff was undertaken.

I have previously stated,"… It is no longer possible to sustain high quality research at the cutting edge of science with the intramural allocations made by the ICMR. We therefore need to seek funds for scientific research from both national and international agencies. Since the competition is intense only the best proposals get funded." I am pleased to place on record that this Centre was able to attract a large cash flow from international agencies as well as the Ministry of Health, Govt. of Tamil Nadu not only for research but also for capacity building. This is a significant development considering the competition for limited funds and will certainly enhance the quality of research output of this Centre.

The power of information technology is being utilised by the Centre as an enabler of change. We inaugurated a website that highlights the past and present research activities of TRC. The major emphasis of our home page is to disseminate all the research findings of the TRC in the past 4 decades. These have remained inaccessible to a large audience because many of them were published in non-indexed journals. The response from the scientific community to this initiative has been overwhelming as seen by the number of hits per week which averages about 4000-5000. During the year the planning for intranet project has been initiated and very soon we will computerise the total activities of TRC. This will facilitate better utilisation of the data that is being collected.

TRC has performed well year after year as seen by its' emphasis on both basic research and applied research and as seen in its numerous publications. As we approach the new millennium we are well primed to better the future. We have acknowledged the need to change drastically and have acted early with redeployment and restructuring of staff of TRC for the newer research strategies. These fundamental changes in the restructuring have been done by participation of all staff members with each one trying their very best to make things happen. The commitment to tuberculosis research and the enthusiasm to adopt the new agenda is reflected in the active participation of the staff in all the activities of the Centre. I am confident that this will be sustained in the years to come so that our dream, of making this institute a global Centre of excellence, will be achieved.

**Dr. P. R. NARAYANAN**
**DIRECTOR.**

# CONTENTS

## GAINING NEW KNOWLEDGE

### Host (immune) response



### Host genetics



### Studies on biology of mycobacteria



## APPENDICES



## LEARNING & SHARING

# STAFF MEMBERS

*(as on 31. 12. 98)*

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P.R. NARAYANAN, *Ph.D.,D.I.I.Sc.*

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| Ranjani Ramachandran, M.B.B.S. | Sr. Research Officer |
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| Sudha Ganapathy, M.A. | Sr. Technical Officer |
| K. V. Kuppu Rao, Ph.D. | Sr. Research Officer |
| Ambujarn Ganesh, D.P.H. | Nursing Officer |
| K. N. Gopilingarn, C.R.A. | Sr. Technical Officer |
| Jayalakshrni Vadivel, BSc. | Sr. Public Health Nurse |
| B. V. S. Chalapathi Rao. BSc. | Sr. Public Health Nurse |
| Arumaikannu Anbalagan, BSc. | Public Health Nurse |
| A. Gunasundari. B.Sc. | Public Health Nurse |
| G. Mangalambal, B.Sc. | Public Health Nurse |
| N. Valarrnathi, B.Sc. | Public Health Nurse |
| Vasanthira Patturaj | Sr. Technical Assistant |
| Santhamma Asokan | Sr. Technical Assistant |
| K. Padmavathy | Sr. Technical Assistant |
| Susan Stella Bai | Technical Assistant |
| K. K. Valasarnma | Clinic Nurse |
| Emily Verghese | Clinic Nurse |
| V. Meenaksni | Clinic Nurse |
| Muthulakshmi | Clinic Nurse |
| Manonmani Nathan | Clinic Nurse |

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| S. Padma | Clinic Nurse |
| Padma Prakash | Clinic Nurse |
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| Annamma Joy | Clinic Nurse |
| S. Chellam | Clinic Nurse |
| R. Mirunalini | Clinic Nurse |
| Victoria Kamalam Jayaraj | Clinic Nurse |
| K. Rosily | Clinic Nurse |
| K. Sampooranam | Technical Assistant |
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| Valliammal Jayaraj | Technical Assistant |
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| C. Arputha Mary | Technical Assistant |
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| G. Kubendiran, M.Sc. | Research Assistant |
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| S. Jagadeesan | Technical Assistant |
| P. Jagannathari | Technical Assistant |
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| T. Nataraj, M.Sc. | Statistical Assistant |
| K. R. Ravichandran, B.Sc. | Technical Assistant |
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| V. Subramanian | Data Entry Operator |
| T. Raman | Data Entry Operator |
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| N. Ravi, DEE., PG Dip.MedEq. | Sr. X-ray Supervisor |
| R. Chandra Mohan, B.Sc. | Sr. X-ray Technician |
| M. S. Govindaraj, B.A. | Dy. Field Supervisor |
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| AbdulKhudoos, B.Com. | Dy. Field Supervisor |
| A. Narasimhan | Dy. Field Supervisor |
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| R. Sashidharan, BSc. | Dy. Field Supervisor |
| B. S. Phaniraj, B.A. | Dy. Field Supervisor. |
| S. Sathiamurthy, B.A. | Dy. Field Supervisor |
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| Annamalai Baskaran, BSc. | Team Leader |
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| K. Somasekar | X-ray Technician |
| S. Dakshinamurthy | X-ray Technician |
| S. Kumar, B.Com. | X-ray Technician |

## Division of Statistics

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| K. G. Fredricks, *B.Sc.* | Statistical Assistant |
| V. Sundaram, *BSc.* | Technical Assistant |
| V. Chandrasekaran, *M.Sc.* | Technical Assistant |
| A. S. Kripasankar, *BSc.* | Technical Assistant |
| R. Segaran. *M.Sc.* | Technical Assistant |
| K. Subramaniam, *M.Sc.* | Technical Assistant |
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| D. Suryanarayanan, *M.Sc.* | Statistical Assistant |
| L. Sekar, *M.Sc.* | Statistical Assistant |
| Shripad Bhat, *M.Sc.* | Statistical Assistant |
| D. Vijaya Bhaskara Rao, *Ph.D.* | Statistical Assistant |
| K. Chandrasekaran, *MSc.* | Statistical Assistant |
| Naik Ashok Chandu, *M.Sc.* | Statistical Assistant |
| C. Ponnuraja, *MSc.* | Statistical Assistant |

## Administration

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| M. Subramanian, *B.Com.* | Sr. Administrative Officer |
| C. J. Arunachalam | Purchase Officer |
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| S.Vasantha | Assistant |
| R. Ramamirtham | Assistant |
| Samuel Swamidoss, *B.A.* | Assistant |
| V. Lakshmanan | Assistant |
| Santhi Velu. *B.A.* | Assistant |

| | |
|---|---|
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| Santha Sriraghavan | Personal Assistant |
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| Kanchana Udayakumar | Sr. T.O. / Recept. |
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| V. K. Venkatesan | Transport Supervisor (Epid.) |
| T. S. Mahadevan | Transport Supervisor (Epid.) |

# STAFF COUNCIL

A Staff Council was constituted on the recommendation of the Director General of ICMR to oversee all activities of the Centre. The Staff Council meets once a month to review all scientific, technical and administrative matters. The proceedings of the Staff Council is disseminated to all staff members. The forum ensures a unique platform for equal participation since Staff Council consists of HODs (Scientific and Administrative Departments), Liaison Officers (SC/ST, OBC) and Women Representatives.

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| Dr. Lalitha Kameswaran | Retd. Vice Chancellor, Tamil Nadu MGR<br>Medical University, Chennai. |
| Dr. H. Srinivasan, | Honorary Editor, Indian Journal of Leprosy,<br>Chennai. |

# Patients' perceptions on public and private providers of TB services

Patients' health seeking behaviour is mainly influenced by their socio-economic conditions, literacy, faith in cure, etc. An attempt was made to understand the user perspective and how the interpersonal and perceptional factors determine the health seeking behaviour.

To identify the reasons for patient preferences for public and private health providers the following study was conducted.

Patients' opinion regarding public and private providers are given below.

| Remarks | Health Providers | |
|---|---|---|
| | Public (153) | Private (85) |
| Positive | • Free treatment<br>• Good care<br>• Better quality drugs<br>• Proper investigation | • Individual attention<br>• Immediate care<br>• Convenient timing<br>• Proximity |
| Negative | • Delayed investigation and treatment<br>• Non-availability of drugs<br>• Lack of guidance<br>• Over crowding<br>• Distance | • Costly<br>• Delayed diagnosis<br>• Non-availability of advanced facility |

A total of 238 patients registered for treatment for tuberculosis with three public health providers (Institute of Thoracic Medicine, Government Royapettah Hospital and Avadi Primary Health Centre) and three private providers (Santhosam Chest Hospital, Sri Ramakrishna Mutt Charitable Dispensary and Diagnostic Centre and Sir Ivan Stedford Hospital) at Chennai were interviewed.

The private sector was utilised by 61% of patients from lower income group (less than Rs.1000/- p.m.) while 42% of the patients being treated by public providers belonged to the above group.

Patient's perception on public and private providers were almost the same on working hours, waiting time and consultation. Patients had longer association with private providers than with public providers.

Among the 153 patients who were taking treatment from public providers 23% had been diagnosed in the private sector whereas only 2% of 85 patients taking treatment at private providers had been diagnosed by public providers. Referral/advice by doctors was the main reason for attending public providers whereas persistence of chest symptoms was the foremost reason for attending private providers.

The study recommends the need for user-friendly attitude of public functionaries and have involvement of private providers in RNTCP.

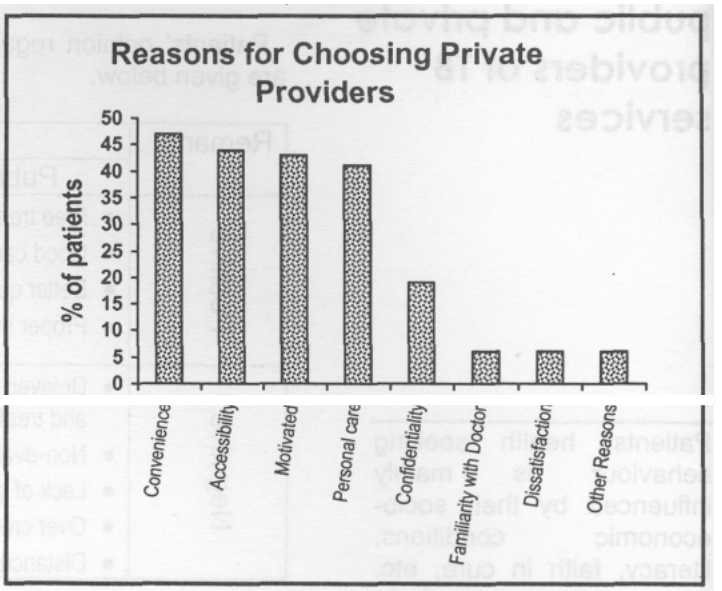
Completed study, *1998*

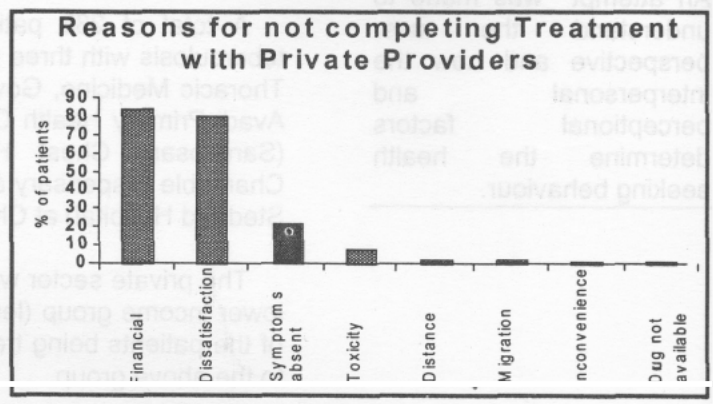
# Sociological profile of tuberculosis patients who had discontinued treatment with private for profit providers

It has been observed that nearly half of the tuberculosis patients approach private sector for treatment. In order to find out their attitude towards private provider pulmonary tuberculosis patients who discontinued treatment fronm the private provider and attending government hospitals were interviewed. This formed part of a WHO funded study aimed to find out the impact of tuberculosis on private for profit providers. These patients were chosen to identify the private providers whom they had approached earlier.

Completed study, 1998

Two hundred and three patients who had discontinued anti-TB treatment with private providers and were attending government health facilities were interviewed.




The multiple reasons for approaching private providers for anti-TB treatment and reasons for discontinuing treatment with the private providers and switching over to government facilities are given in the figures. While convenience and accessibility are the reasons for choosing private providers cost, of treatment with them is the main reason for changing over. Eighty-five percent of patients preferred to go back and continue anti-TB treatment with the private providers if the drugs were given free of cost.

This brings out the need to involve private providers in the control programme and provide drugs free of cost through them.

# Treatment compliance in relation to sources of referral and initial reaction to the diagnosis of tuberculosis

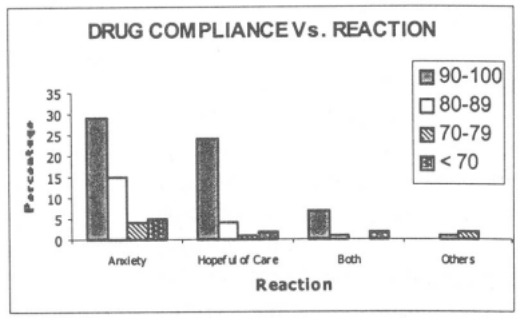
Case finding in tuberculosis is passive and aims to diagnose and treat the chest symptomatics who attend the health facilities. It is important to identify the influencing factors or groups within a community that influence a symptomatic to seek treatment. An understanding of the initial reactions of patients to the diagnosis could perhaps help in predicting their compliance.

Completed study, 1998

One hundred and seven newly diagnosed sputum positive patients attending the Centre were questioned regarding their initial reactions to the diagnosis and as to how they reached a specialized Tuberculosis Research Centre for diagnosis and treatment. Drug compliance of these patients in relation to sources of referral are given in table. Patients' initial reactions to diagnosis in relation to their compliance is brought out in the figure.

Drug compliance (vs) Referrals

| Drug Compliance (%) | Referred by | | | | | TOTAL | |
|---|---|---|---|---|---|---|---|
| | Family | Friends/ Neigh. | NGO | Pvt. pract. | Own | No. | % |
| 100 | 6 | 2 | 3 | 6 | 6 | 23 | 21 |
| 90-99 | 13 | 17 | 9 | 4 | 4 | 47 | 44 |
| 80-89 | 6 | 7 | 2 | 4 | 2 | 21 | 20 |
| 70-79 | 2 | 1 | 2 | 2 | 0 | 7 | 7 |
| <70 | 4 | 2 | 0 | 3 | 0 | 9 | 8 |
| ALL n(%) | 31 (29) | 29 (27) | 16 (15) | 19 (18) | 12 (11) | 107 | 100 |




Since the family, neighbours, private practitioners have an influence on referrals those groups can be targeted to increase their awareness on tuberculosis so that they can facilitate case finding. It is unfortunate that the initial reactions to the diagnosis is more negative than positive

The influence of the family members and friends in referring symptomatics for investigations has been considerable . This reflects the sense of responsibility existing within the country which should be exploited in the treatment of a disease such as tuberculosis and therefore calls for creating more awareness in the community.

## Tuberculosis - Facts

- Estimated 140 lakh people have tuberculosis infection.
- Estimated 35 lakhs are sputum positive.
- About 22 lakh new cases every year including about 10 lakh sputum positive.
- 5 lakh people in India die from TB every year, more than 1000 every day.
- One sputum positive case can infect 10-15 healthy individuals in one year.

# Feasibility of utilising literate youth volunteers to improve nutritional status of antenatal and postnatal women and children under five years in a tribal area Jawadhu Hills

Jawadhu Hills is a tribal area in the North Arcot District of Tamil Nadu. It is a hilly terrain with widely scattered hamlets and a total population of one lakh. During a previous study, we evaluated the feasibility of utilising tribal literate youth volunteers for case finding and case holding in tuberculosis. During that period, there was a demand from the tribal population for comprehensive health care delivery. We had also observed a high incidence of malnutrition among young children and women of child bearing age. Since we had a willing and trainable work force drawn from the community available to us, we decided to evaluate the feasibility of utilising them for delivery of health care services.

Completed study, 1998

The Tamil Nadu Integrated Nutrition Project (TINP) is a supplementary feeding program targeted at antenatal and postnatal women and children in the age group of 6 mths to 3 years. It involves regular weighing (growth monitoring) of children in this age group and giving supplementary food to those who are in Grades II, III or IV malnutrition. The program functions through Community Nutrition Centres which are established for a population of 1000. Since the hamlets in tribal areas generally have 10 to 20 households only and their population is less than 1000 usually, there are no CNCs functioning. In Jawadhu hills, there was only one CNC in Jamunamaruthur, the main town. Villagers have to walk 10 to 15 km to reach this town, so children from outside the area could not attend or utilise the CNC.

We decided to collaborate with TINP in providing nutritional services in this difficult terrain. The use of trained community volunteers could serve as a model for delivery of other health care services also and could be replicated in other areas of the country if found to be effective.

i. To assess the feasibility of using tribal literate youth to improve the nutritional status of children under five years and antenatal and postnatal women
ii. To determine the extent of undernutrition among tribal children and antenatal women in this tribal area

The study was designed and performed in stages as detailed below :

1. Training of TRC staff at TINP: Three Medical Officers, 2 Social Workers and 5 Paramedical staff underwent an intensive 10 day training program at TINP in techniques of nutritional evaluation, assessment and intervention.
2. Census and enumeration of study population by literate youth: Sixty volunteers (literate boys and girls), 1 from each village were identified. The volunteers were trained in techniques of census taking, enumerating and recording. The census was taken of the entire population and the details of children under five year and antenatal women were recorded separately. The study population was thus identified.
3. Baseline nutritional survey of children under 5 years: The children identified in the census were examined. Height, weight and mid-arm circumference were recorded. Weight of all antenatal women in the study area was recorded. This was repeated every month by the youth volunteers.

4. Baseline investigations (Hb, stool): Hb estimation and stool examination for ova and cysts was performed bv a laboratory technician from TRC on a sample of children from the study area.

5. Training of literate youth: The literate youth were trained by TRC staff in the field and monitored closely. Random checks were performed to evaluate their technique of weighing. They also underwent a formal 2 week training in nutritional techniques at TINP in January, 1997.

6. Intervention

a) Supplementary feeding based on TINP pattern: Children were weighed every month and their growth monitored using standard growth, charts. All children in the age group 6-36 months who were in Grades II, III or IV malnutrition were given supplementary (weaning) food supplied by TINP. The food packets were handed over to the concerned volunteers (from that village) who supervised the feeding of malnourishd children in his/her care. All antenatal women (from 6 months of pregnancy onwards) till 6 months after delivery (postnatal) were given the supplementary food. Supplementary feeding was started in January 1997.

b) Regular deworming: All children in the age group 1-5 years were dewormed using single dose Albendazole once in 6 months.

c) Vitamin A supplementation: One lakh Units of Vit-A was administered to all children in the age group 1 - 5 years, once in 6 months.

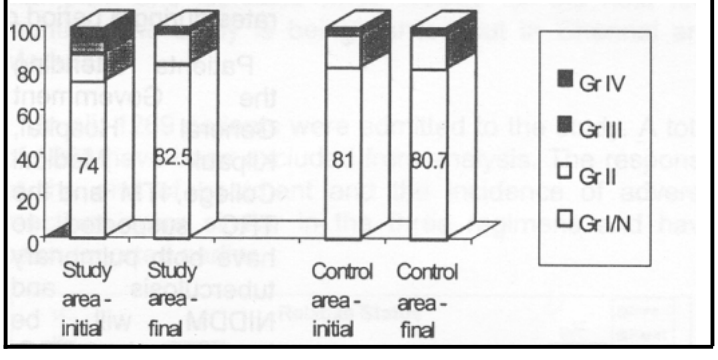
d) Iron, folicacid and calcium supplementation was given to all antenatal women from the 6th months

e) Health education of population: This was done by organising village meetings, addressing small groups of mothers, screening of films on nutrition (by TINP) and one to one interaction with the villagers during our visits. Stress was laid on general and personal hygiene, care of infants and young children and their nutritional requirements and the importance of immunisation.

7. Final round of nutritional assessment of the study population: The entire cohort (study population) was examined again in September 1997, March 1998 and September-October 1998. Height, weight and mid-arm circumference was measured for all the children. Hb estimation was repeated on a sample of children.

8. Control area: A similar number of children in the age group 0-5 years from the same tribal area were included as controls. Weight was recorded in July 1997 and again in July 1998. No visits were made or intervention carried out in the intervening period.

PREVALENCE OF MALNUTRITION IN CHILDREN UNDER 5 YEARS




There were 1576 children (833 boys and 743 girls) in the age group 0-5 years in 60 study villages, registered in October 1996. These were followed up as a cohort upto September 1998 and children born during this period were also included in the group. Figure above1 shows the prevalence of different grades of malnutrition in the study and control groups (children 0 - 5 years) at two different time points. It was observed that the prevalence of severe grades of malnutrition had decreased from 12% to 2.4% in the study area while there was no change in the control area.

1. The prevalence of malnutrition was high among children under five years initially (Grades II, III and IV malnutrition of 25.5%) in the Jawadhu Hills tribal area.
2. It is possible to train community volunteers to deliver nutritional services and they are well accepted by the
3. Due to the supplementary feeding and health education of the community, the prevalence of severe grades of malnutrition (Grades III,IV) in children aged 0-5 years has decreased from 12% to 2.4%. This model of health care delivery may be replicated in other settings.

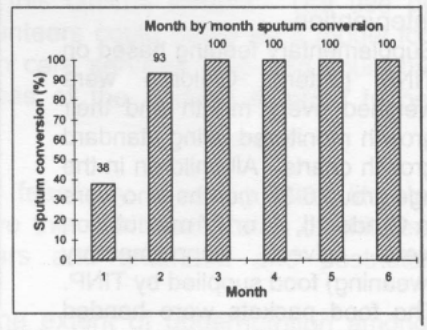
# A study of the category I regimen (RNTCP) for the treatment of pulmonary tuberculosis associated with non-insulin dependent diabetes (NIDDM)

Pulmonary tuberculosis has been reported to be approximately twice as common among diabetics as among non-diabetics. Active tuberculosis intensifies diabetes mellitus; thus the two diseases constitute a dreaded combination.

Ongoing study, 1998-2003

Recent surveys in India indicate that the prevalence of tuberculosis among symptomatics varies from 4.4 to 11.0 per 1000 and it is rising from 10% to 25% by the age of 60 years as per a WHO report. Similarly, non-insulin dependent diabetes (NIDDM) is also more common among older subjects. Studies conducted in TRC had shown that short-course regimens of 6 to 9 months have been found to be effective both in pulmonary and extra-pulmonary forms of tuberculosis. It has been reported from Japan (retrospective analysis) that a 12-month "short-course chemctherapeutic" regimen was effective in diabetic patients with pulmonary tuberculosis. Under Revised National TB Control Programme Category I regimen ($2EHRZ_3/4RH_3$) is recommended for patients with DM and hence it is proposed to assess the efficacy of the above 6-month regimen, both cure rate and relapse rates during a period of 3 years from admission to study.

Patients attending the Government General Hospital, Kilpauk Medical College, ITM and the TRC suspected to have both pulmonary tuberculosis and NIDDM will be investigated at TRC. It is proposed to admit 60 patients to the study.




All patients will receive the following regimen: $2EHRZ_3$ /$4RH_3$ – Ethambutol 1200 mg plus isoniazid 600 mg with pyridoxine 10 mg plus rifampicin 450 mg for patients weighing less than 60 kg and 600 mg for patients weighing 60 kg or more plus pyrazinamide 1.5 g for all patients three times a week under supervision for 2 months followed by rifampicin plus isoniazid in the same dose for the next 4 months. However, stratification will be done according to the smear grading and $HbA1_c$ levels.

A total of 21 patients were admitted to pilot study of whom 17(81%) were males. The mean age was 49 years (range: 34-70 yrs.) and the mean body weight was 48.4 kg. (range: 32.6-60.0 kg.).

Among the 17 patients who had completed a full course 6 months of treatment, 2 were lost (both migrated to a village one after 1 month of treatment and one during the first month of treatment). Of the remaining 15 patients all had received eighty percent or more of chemotherapy and all became negative by culture for *M. tuberculosis* from 3rd month onwards (Fig.). The main study is funded by Anti-Tuberculosis Association of Tamil Nadu and is in progress.

## Six month regimens of treatment for pulmonary tuberculosis with two double-drug combinations on alternate days in the intensive phase- follow-up phase

Several highly effective chemotherapy regimens of 6-8 month duration are available for the treatment of pulmonary tuberculosis. Most of these regimens employ, four drugs, namely, isoniazid, rifampicin pyrazinamide and ethambutol in a single dose, either daily or thrice or twice a week in the intensive phase. The number of tablets/ capsules to be consumed by the patient is thus large and some patients find this difficult and experience adverse reactions. If the 4 drugs can be split into two portions and given on alternate days, and if the efficacy of such a regimen can be established, it would have the advantage of better acceptance to patients because of possibly fewer side effects.

Ongoing study, 1995,-2000

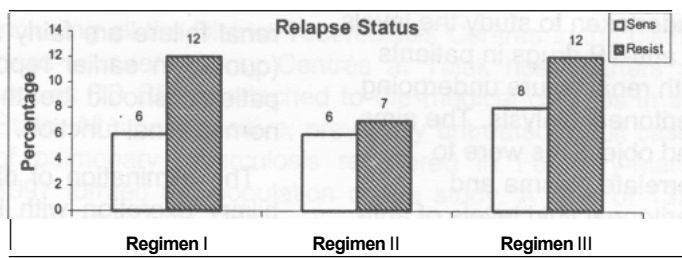
To address this question, the Centre is carrying out a randomised controlled clinical trial to assess the efficacy of the following regimens in the treatment of sputum positive pulmonary tuberculosis patients.

Regimen-I: Rifampifin and ethambutol on 1 day and isoniazid and pyrazinamide on the next day alternately, for two months, followed by rifampicin and isoniazid twice weekly for the next four months.

Regimen-II: Rifampicin and ethambutol on 1 day and isoniazid and pyrazinamide on the next day alternately, for three months, followed by rifampicin and isoniazid twice weekly for the next three months.

Regimen-III: Rifampicin, isonaizid, ethambutol and pyrazinamide thrice weekly for two months followed by rifampicin and isoniazid twice weekly for the next four months. The study is being carried out in Chennai and Madurai.

In all, 1269 patients were admitted to the study. A total of 1999 have been excluded from analysis. The response at the end of treatment and the incidence of adverse reactions were similar in the three regimens and have been reported earlier.




Relapse: A patient was considered to have relapsed if during follow-up, at least two sputum specimens in a two month period yielded *M.tuberculosis* on culture, at least one of which have 20 colonies or more, and if one sputum smear was positive. A total of 938 patients who successfully completed treatment have been followed up for 18 months; 808 had fully drug sensitive organisms initially, and 130 had resistance to one or more drugs.

Of the 808 drug sensitive patients, 16 (6%) of 267 patients in Regimen-I, 17(6%) of 290 patients in Regimen-II, and 19 (8%) of 251 patients in Regimen-III had bacteriological relapse.

Of the 130 drug resistance patients, 5 (12%) of 42 patients in Regimen-I, 3 (7%) of 46 patients in Regimen-II, and 5 (12%) of 42 patients in Regimen-III had bacteriological relapse. These differences were not statistically significant. All the patients who relapsed were retreated.

# Pharmacokinetics of isoniazid and rifampicin in patients with renal failure undergoing continuous ambulatory peritoneal dialysis (CAPD)

Investigations were undertaken to study the levels of anti-TB drugs in patients with renal failure undergoing peritoneal dialysis. The aims and objectives were to correlate plasma and peritoneal fluid levels of anti-tuberculosis drugs namely INH and RMP.

Completed study, 1996-98

Out of 22 patients admitted to the study 13 were slow acetylators. Among these patients, 7 were on CAPD and 6 were on NDPD. Among 9 rapid acetylators, 4 were on CAPD and 5 were on NDPD. It needs to be mentioned here that for 1 patient on CAPD, the exposure calculated based on the plasma of isoniazid was very low in spite of the half-life being >2.71 ($t_{½}$--3.44, AUC 0-8 h. 27.23 μg/ml.h.) and has been included in the rapid acetylator group.

Table below gives the pharmacokinetic variables calculated based on the levels of INH and RMP in plasma, respectively. There are no differences between the patients on LMP and HMP in pharmacokinetic variables, such as $C_{max}$, $T_{max}$ and AUC. But, there is three-fold increase in the elimination half-life for both the drugs when the results are compared with the findings on the healthy volunteers reported earlier.

Thus, the findings have shown that in daily regimens containing isoniazid, it may be advisable to prescribe a lower than the standard dosage of isoniazid to slow acetylators with renal failure, especially, to light-weight patients. However, the dosage should not be too low (not below 5 mg/kg in any case) as that would reduce the peak concentration with which drug efficacy is related. Since plasma concentrations in rapid acetylators with renal failure are fairly similar to those of healthy subjects (quoted in earlier report), the dose of isoniazid in these patients should be the same as it is for patients with normal renal function.

The elimination of rifampicin is mainly through hepato-biliary excretion with kidneys playing only a minor role. Even in healthy subjects, only about 10-15% of the dose administered is excreted as the drug and its primary metabolite in urine over a 24-hour period.

The findings reported in this study have shown that because of the three to four fold increase in the half-life, with regard to rifampicin levels, the dosage of rifampicin also needs to be reduced for patients with renal failure undergoing peritoneal dialysis as it is for isoniazid in slow acetylators.

**Pharmacokinetic variables based on plasma levels**

| P.V. | INH Slow | | INH Rapid | | RMP | |
|---|---|---|---|---|---|---|
| | LMP (n=8) | HMP (n=6) | LMP (n=3) | HMP (n=5) | LMP (n=11) | HMP (n=11) |
| **Peak Conc. (μg/ml)** | 11.73 (19.30-15.51) | 11.71 (7.82-13.41) | 7.17 (5.08-10.64) | 10.43 (4.18-18.80) | 10.37 (4.89-18.31) | 12.86 (2.48-37.13) |
| **Coverage (h.)** | 39.64 (18.24-57.62) | 37.4 (30.92-42.72) | 12.04 (10.62-14.53) | 14.67 (13.33-16.58) | 42.33 (15.87-74.90) | 43.00 (21.32-119.03) |
| **Exposure (μg/ml.h.) (0-8)** | 69.09 (49.04-89.05) | 61.17 (49.79-72.25) | 22.65 (12.85-32.81) | 34.49 (18.55-62.87) | 56.49 (21.57-102.95) | 63.30 (11.67-187.35) |
| **$T_{½}$** | 8.16 (6.31-9.08) | 6.15 (4.77-7.38) | 2.42 (1.66-3.44) | 2.46 (2.29-2.61) | 9.19 (3.00-16.51) | 9.52 (5.01-29.42) |

# WHO-sponsored surveillance of drug resistance in tuberculosis in Tamil Nadu, South India

This study, as part of the WHO/IUATLD global project on drug resistance surveillance in over 40 countries was undertaken in February-March 1997 in collaboration with the Government of Tamil Nadu.

The aims of this study were:

a) To determine the prevalence of initial and acquired drug resistance in the survey area in order to use the information as a prevalence indicator for the National Tuberculosis Programme and to assess whether the recommended regimens are adequate.

b) To establish the foundation for routine surveillance of drug resistance with procedures based on defined guidelines in order to observe trends in drug resistance in the survey area.

c) To promote external and internal quality control on laboratory procedures of susceptibility testing, in collaboration with Supranational Reference Laboratories.

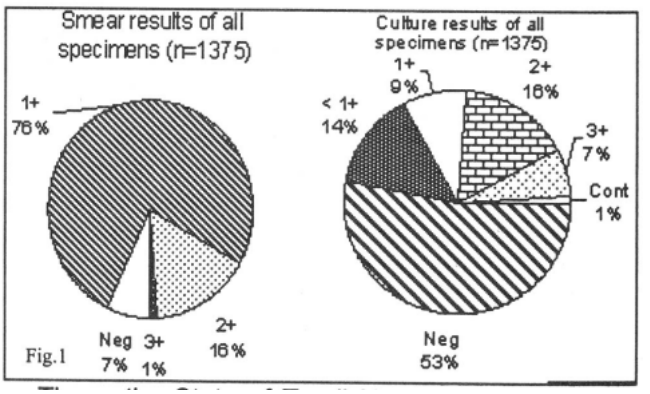


Fig.1

The entire State of tamil Nadu formed the survey area. A total of 145 centres participated in the survey including all the District Tuberculosis Centres in the district head-quarters, X-ray Centres at Taluk headquarters as well as TB Clinics attached to the medical colleges in the State. All smear-positive, previously untreated, adult cases of pulmonary tuberculosis registered in February-March 1997 formed the population of the study. A total of 1375 specimens from 713 patients was collected in sterile containers with 3 ml of 1% cetyl pyrindinium chloride. the specimens were transported and processed for smear culure (by Pertroff's method), drug susceptibility as well as identification tests.

Of the 1375 specimens, 90 (7%) were found to be smear-negative (Fig.1). Further, there was an excellent agreement between duplicate smear results from the same patient (Fig.2), the interpatient agreement being 87%.

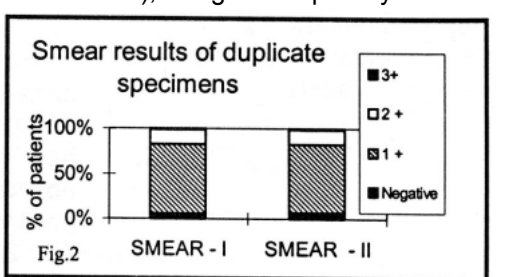


Fig.2

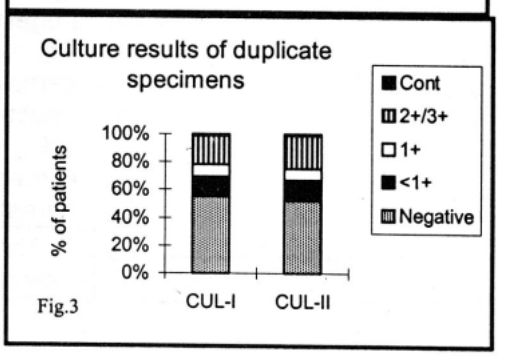


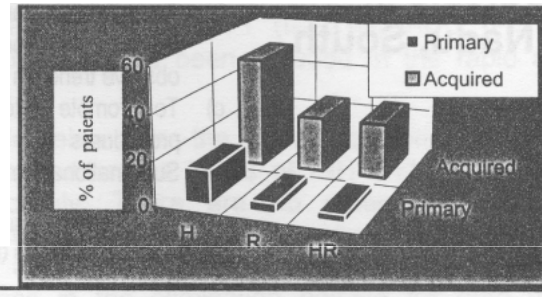
Fig.3

Completed study, 1997-98

The culture results of all the specimens, however, revealed that as many as 742 (53%) were culture negative (Fig.3). A similar pattern was observed when duplicate specimens from the same patient were considered (Fig.4). This high percentage of culture negativity from smear-positive specimens was observed to be due to a faulty culture procedure in the protocol recommended.

The drug susceptibility results were available for 400 patients, comprising of 384 patients with no history of previous treatment and 16 patients with previous treatment. Resistance to isoniazid, alone or in combination with other drugs, was observed in 15.4% of the former and in 50% of the latter.

Any resistance to rifampicin was observed in 4.4% of untreated patients as against 25% of patients with previous treatment. These included 3.3% of patients with H & R resistance in the untreated group and 25% in the treated group.

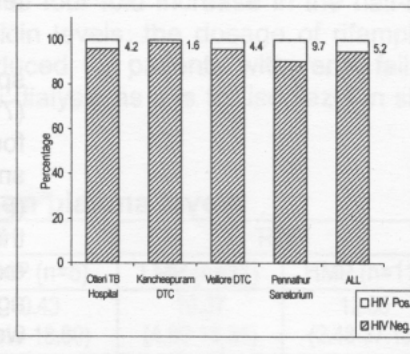



This study made available , for the first time, authentic basic data on initial and acquired drug resistance from Tamil Nadu. Further studies are also in progress for evolving a proper culture methodology in CPC-containing specimens and to recommend a modified protocol for future surveillance studies involving transportation of specimens containing cetyl pyrindinium chloride as a preservative.

# Seroprevalence of HIV Infection among TB patients

The study was planed in order to (i) find the seroprevalence rate of HIV infection among TB patients and (ii) to study the trend of HIV infection among TB patients in Vellore.

All newly diagnosed TB patients attending the 4study centres namely, the Otteri TB hospital, Chennai; DTC Kancheepuram;DTC,Vellore and Pennathur Sanatorium Vellore were registered and detailed history and clinical examination findings were recorded.The Medical Officer, TB Clinic categorised the patients based on previous treatment history, sputum smear results and other investigations. After obtaining informed consent, 3ml venous blood and 2 sputum specimens were collected from each patient. In all, 3260 patients were registered upto December 1998. The HIV seropositivity rates from the different centres are shown in the figure below.




Among the 168 seropositives from 3260 screened, 42% had newly diagnosed sputum smear positive pulmonary TB, 23% had smear negative pulmonary TB, 15% were defaulters, 4% were treatment failures, 3% had extra-pulmonary TB, 2% had relapse requiring retreatment and 10% others.

The HIV Seroprevalence rate among TB patients in Vellore has risen from about 2% in 1993 to 7.4% in 1998.

Completed Study 1997-98

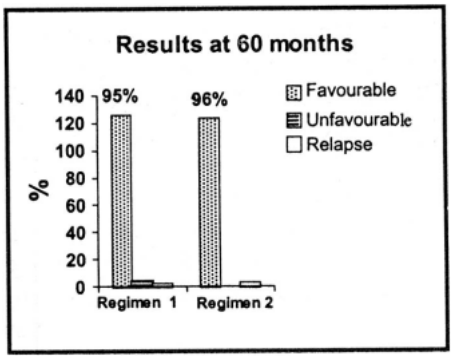
## Collabarative controlled clinical trial on treatment of lymphnode tuberculosis

Tuberculosis of the lymphnodes is the commonest form of extra pulmonary tuberculosis, constituting about 10% of all cases of tuberculosis. In terms of morbidity, therefore, a significant number of patients suffer from this form of the disease. While the treatment of pulmonary tuberculosis has been systematically studied over the years, the same is not the case for lymphnode tuberculosis It is therefore necessary to evolve standardised treatment regimens for lymphnode tuberculosis This study was designed to address such a need.

Completed study, 1988-98

This was a randomized controlled clinical trial on treatment of lymphnode tuberculosis, and was conducted by the TRC Unit at Madurai in collaboration with the Pediatric and Adult Surgery Departments of the Government Rajaji Hospital. Patients with biopsy confirmed superficial lymphnode tuberculosis were randomized to either of the two following 6 month regimens of treatment, viz. Regimen 1: 6RH daily; an unsupervised daily regimen of isoniazid and rifampicin for six months, the drugs being supplied twice a month for self-administration, or Regimen 2: 2RHZtw/4RHtw: a supervised twice weekly regimen of isoniazid and rifampicin for six months with pyrazinamide for the first two months. All patients were reviewed monthly up to 12 months, every three months up to 24 months and every six months up to 60 months.

The study concluded in September 1998. Two hundred and seventy seven patients were admitted to the study. Patient characteristics and the results at the end of treatment were presented in the Annual report of 1993. After excluding 15 patients, 116 (87%) of 133 patients in Regimen 1 and 112 (87%) of 129 patients in Regimen 2 had a "Favourable" response at the end of treatment; 13 (10%) patients in Regimen 1 and 17 (13%) in Regimen 2 had "Doubtful" response, i.e., had palpable lymphnodes exceeding 10 mm diameter. Four (3%) patients, all treated with Regimen 1 had an "Unfavourable" response and had their treatment changed.




Patients have now been followed up for 60 months. During this period, of the 13 patients in Regimen 1 who had a "Doubtful" response at the end of treatment, in 12 the nodes regressed or disappeared subsequently and the response was reclassified as "Favourable", the other relapsed. In addition, one patient relapsed in Regimen 1 from the initially "Favourable" category. In Regimen 2, of the 17 patients with "Doubtful" response at the end of treatment, 15 were reclassified as "Favourable", one relapsed and one still had large nodes at 60 months. In addition two patients relapsed from the initially "Favourable" category. Two patients died due to non-tuberculous causes, one from each regimen.

In summary, after 60 months of follow-up of 262 patients treated with two six month regimens of treatment for superficial lymphnode tuberculosis, 126 (95%) of 133 patients treated with an unsupervised daily regimen (6RH daily), and 124 (96%) of 129 patients treated with a supervised twice-weekly regimen (2RHZtw/4RHtw) had favourable outcomes. Four patients treated with Regimen1 failed to respond to treatment. Two patients relapsed in Regimen1 and three in Regimen2. There was no difference in the results in children and adults.

This study has shown that both the six month treatment regimens investigated were very successful, with high cure rates and low relapse rates.

## Collaborative clinical study of cutaneous tuberculosis - Follow up phase

A collaborative clinical study of cutaneous tuberculosis with an aim to evolve diagnostic criteria and to assess the efficacy of an SCC regimen of 9 month duration (from April 92 to Dec.96) and 6 month duration (from Jan.97 to Dec.98) is being carried out at the Centre.

Ongoing study, 1992-1999

Patients diagnosed clinically as having cutaneous tuberculosis by the dermatologist are admitted to the study after a skin biopsy. Patients are treated with rifampicin (450mg) and isoniazid (300mg) daily for 9 months/6 months and those aged less than 12 years are treated with weight adjusted dosages. The patients are assessed at the Centre and also at the collaborating hospitals every month during treatment at 3-monthly intervals upto 24 months and 6-monthly intervals upto 36 months. After our interim findings the duration has been reduced to 6 months from January 1997.

A total of 289 (249 : 9HR - 40 : 6HR) patients were admitted to the study and 288 have completed chemotherapy. After excluding 64 patients (Non-TB 22, HIV 2, Change of Rx. 4, Non-TB death 2, Lost after admission 34) there remains 224 (193 in 9HR, 31 in 6HR) in the analysis. A total of 182 (81%) patients had single lesion, 30 (13%) had 2 lesions and 12 (5%) had multiple lesions.

The diagnosis of cutaneous tuberculosis was confirmed by bacteriology and/or histopathology in 201(90%) of 224 patients.

Overall resolution was observed in 193 (86%) patients. The lesion resolved at the end of 6 months in all types. However, at the end of 9 months over all resolution was 213 (95%). One patient with lupus vulgaris, 1 patient with verrucosa cutis and 1 patient with scrofuloderma did not respond to the prescribed regimens. So treatment was changed and all 3 patients are responding well. In the remaining 8 patients (all verrucosa cutis in 9HR) the induration subsided subsequently but verrucosity persisted which disappeared after applying keratolytic agent without specific anti-tuberculous treatment..

## Controlled clinical trial for the treatment of sputum positive pulmonary tuberculosis with regimens containing ofloxacin

In vitro studies in TRC have shown that ofloxacin, a member of the fluoroquinolone group of drugs, has mycobactericidal activity equal to that of rifampicin and isoniazid. An earlier study conducted at this Centre had shown that a 3-month regimen of streptomycin, isoniazid rifampicin and pyrazinamide daily (3SHRZ) was near 100% effective in patients with initially drug-sensitive organisms. However 16.8% of these had a bacteriological relapse over a 5-year follow-up period. Based on this information, the Centre is currently undertaking a study to find out if the duration of treatment for smear positive pulmonary tuberculosis could be reduced to 3, 4 or 5 months by using ofloxacin along with other drugs in the treatment regimens.

The minimum duration of treatment for sputum positive pulmonary tuberculosis is six months. Even with a six month short course regimen the treatment completion rate under programme conditions is only around 50%. There is a need therefore to further shorten the duration of treatment to ensure better compliance.

This is a randomized controlled clinical trial being conducted in Chennai and Madurai. Patients with sputum positive pulmonary tuberculosis with previous chemotherapy not exceeding 2 weeks, are randomly allocated one of the following 4 regimens.

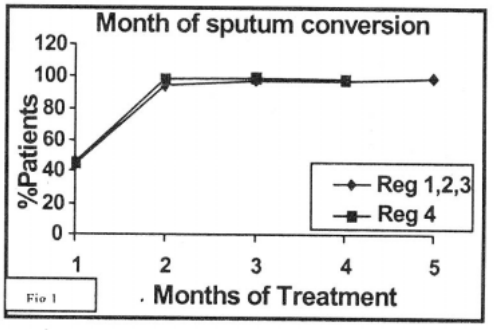


Fig 1

Regimens:

1. 3OHRZ/- *(ofloxacin, isoniazid, rifampicin and pyrazinamide daily for three months)*
2. 3OHRZ/1RHtw *(same as in Regimen 1, followed by rifampicin and isoniazid twice a week for one month)*
3. 3OHRZ/2RHtw *(same as in Regimen 1, followed by rifampicin and isoniazid twice a week for two months)*
4. 2OHRZ/2RHtw *(ofloxacin, isoniazid, rifampicin and pyrazinamide daily for two months, followed by rifampicin and isoniazid twice a week for two months)*

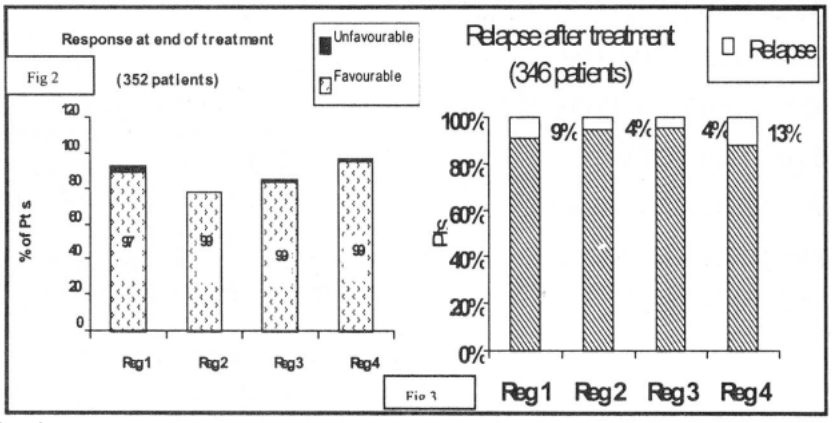
All drugs are given under supervision at the Clinic. Patients who default for treatment are visited at home and motivated to attend. It is proposed to follow up the patients for five years.

Intake to the study was completed in December 1998 A total of 529 patients have been admitted to the study. Interim analysis in 352 patients have shown that at the end of treatment, overall 97 to 99% of the patients had a favourable response (see figure). Forty-two patients had pre-treatment resistance to isoniazid, other than one of whom had a favourable response (Fig 2). Six patients had resistance to rifampicin and isoniazid (MDR Tuberculosis), one of whom was also resistant to ofloxacin. Of these two patients had an unfavourable response, one relapsed and the other three had favourable response.

Ongoing study, 1995-2003

The proportion of patients becoming culture neagative at the end of two months of treatment is a measure of the bactericidal activity of the regimen. After amalgamating the results of regimens 1,2 and 3, all of which had an intensive phase of three months and comparing it with the fourth regimen, which had an intensive phase of only two months, it was found that the rate of culture conversion were very similar. Culture conversion at two months ranged from 94 to 97%.

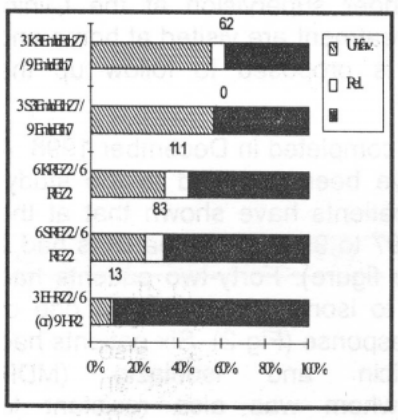
Relapse rates after successful completion of treatment is a measure of the sterilizing activity of the regimens.




showed that the relapse rates were 9%, 4%, 4% and 13% respectively in the four regimens (Fig.3) Even though the relapse rates in Regimen 1 and 4 are higher compared to Regimens 2 or 3, the difference was not statistically significant

Interim analysis has thus shown that the regimens under study have yielded good results at the end of treatment with practically all patients showing a favourable response. However a longer period of follow-up is necessary to get relapse rates before arriving at conclusions on the efficacy of the regimens.

## Treatment regimens for patients who fail or relapse on Short Course Chemotherapy : Follow-up phase




Ongoing study, 1987-2000

Pulmonary tuberculosis patients who have been treated with short course regimens and who (i) show a serious clinical deterioration, (ii) have a persistent radiographic deterioration, (iii) have an unfavourable bacteriological response or (iv) have a bacteriological relapse requiring retreatment are prescribed an appropriate regimen depending on the last available drug sensitivity test results.

The chemotherapeutic regimens are as follows:

1 $3EmbHRZ_2$ / 6 / $9RH_2$ for patients with organisms sensitive to rifampicin and isoniazid.

2(a) $6SREmbZ_2$/ $6REmbZ_2$ for patients with organisms resistant to isoniazid.

(b) $6KREmbZ_2$/ $6REmbZ_2$ for patients with organisms resistant to streptomycin and isoniazid.

3(a) $3S_3EmbETHZ_7$ / $9EmbEthZ_7$ for patients with organisms resistant to rifampicin and isoniazid.

(b) $3K_3EmbEthZ_7$ / $9EmbEthZ_7$ for patients with organisms resistant to rifampicin, streptomycin and isoniazid.

The salient findings are presented in the figure on treatment outcome. The intake to the study has been completed and the patients are on follow-up.

# Action of metronidazole in combination with isoniazid and rifampicin on persisiting organisms in experimental murine tuberculosis

It is well recognised that tubercle bacilli have the ability of sequestering themselves to stay in a dormant state in the host. None of the currently used anti-tuberculosis drug regimens completely eliminate this population and the possibility of endogenous reactivation due to a small populatoin of "dormant" or "persisting bacilli" at a later date always remains. We used a murine model to simulate dormant condition as described in the "Cornell model" and studied the effect of metronidazole on presumably "persisting" bacilli.

Completed study, 1994-98

BALA/c mice were infected with *M.tuberculosis* and, after 14 days, treated with isoniazid (H) or rifampicin (R) or isoniazid plus rifampicin (HR) for 2 months. An untreated group and a group treated with metronidazole (M) alone served as controls. At the end of 2 months, M was added to the H, R and HR regimen in half the mice,

Table 1: $Log_{10}$ colony forming units of *M.tuberculosis* in lung

| Group | Months after start of treatment | | | 3 Months after stop Rx. |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Control* | 7.19 (1.02) | 5.3 (0.54) | 3.88 (0.47) | 5.24 (0.73) |
| M | 5.34 (1.45) | 6.44 (0.88) | 4.88 (0.41) | 3.71 (0.34) |
| H | 3.88 (0.33) | 1.64 (0.67) | 0.67 (0.54) | 3.40 (1.40) |
| R | 0.98 (0.80) | 0.97 (0.79) | 1.84 (0.79) | 1.09 (0.09) |
| HR | 2.12 (1.03) | 1.00 (0.58) | 0 (0) | 1.98 (1.14) |
| HM | | @ | 1.04** (0.85) | 1.83 (1.50) |
| RM | | @ | 0** (0) | 2.50 (0.86) |
| HRM | | @ | 0** (0) | 1.34 (1.09) |

* At 2 weeks after infection, the mean $log_{10}$ cfu was 5.00.
@ Same values as for isoniazid (H), rifampicin (R) and HR respectively, since metronidazole was not added at these time points.
** 1 month after start of metronidazole (M). Values in bracket indicate standard error of the mean.

and the treatment was continued for one more month in all mice. The drugs were administered by oral gavage in 0.2 ml of 0.1% agar. The dosages were as follows: M 100 mg/kg, R 10 mg/kg, and H 25 mg/kg.

In the lung and spleen of infected mice 5.00 and 4.95 $log_{10}$ CFU of *M.tuberculosis* were present at the start of treatment. At the end of the 2nd month of treatment, the $log_{10}$ CFU of *M.tuberculosis* in lung was the lowest in mice treated with R alone (0.97) followed by HR (1.00) and H (1.64) (Table 1). In spleen, the corresponding values were 1.09, 0.67 and 2.07, respectively (Table 2). Compared to the untreated control group, there was no reduction in $log_{10}$ CFU of mice treated with M alone, either in lung or spleen.

At the end of the 3rd month of treatment, in the lung of mice treated with R alone the $log_{10}$ CFU of *M. tuberculosis* increased to 1.84 from the 2nd month count of 0.97. This was because *M.tuberculosis* was isolated from the lungs of 2 out of the 3 mice sacrificed in the R group after the 3rd month of treatment. On drug susceptibility

testing of these strains, one was found to be resistant to R (MIC >128) and the other was sensitive. At this time point, *M. tuberculosis* could not be isolated from the lungs of any of the 3 mice which were treated with M in addition to R(RM group). The $log_{10}$ CFU in the lungs of mice treated with H and HM were 0.67 and 1.04, respectively, at this time point. In spleen, the $log_{10}$ CFU of *M.tuberculosis* was lower in the RM group (0.97) compared to R group (1.49); and again in the HM group (1.13) compared to the H group (1.80). None of the above differences were statistically significant. In both lung and spleen, no detectable count was obtained at the end of 3 months in mice treated with HR and HRM.

At 3 months after stopping treatment, *M.tuberculosis* was isolated from both the lung and spleen of all the groups, including the groups in which *M.tuberculosis* was undetectable at the end of 3 months of treatment (tables 1&2). However, in these groups, the $log_{10}$ CFU of M.tuberculosis was lower in groups with M compared to the respective single or two-drug groups in both lung and spleen, except in the RM in which the $log_{10}$ CFU in the lung was 2.50 compared to 1.09 with R alone.

In the present study, metronidazole showed an additive effect when combined with rifampicin in reducing the $log_{10}$ CFU of *M.tuberculosis* in both the lungs and spleen at the end of 3 months of treatment. When metronidazole was combined with isoniazid this effect was

Similar observations were reported earlier by Wayne & Sramek in their *in vitro* experiment (Wayne L.G. and Sramek H.A. Antimicrob. Agents Chemother. 1994; 38: 2054-8).

Table 2: $Log_{10}$ colony forming units of *M.tuberculosis* in spleen

| Group | Months after start of treatment | | | 3 Mths. after stop Rx. |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| Control* | 7.17 (0.91) | 4.49 (0.40 | 3.48 (0.35) | 3.47 (0.42) |
| M | 5.98 (1.15) | 5.73 (1.21) | 3.78 (0.28) | 3.13 (0.35) |
| H | 4.52 (0.13) | 2.07 (0.84) | 1.80 (0.73) | 3.19 (0.29) |
| R | 2.39 (0.23) | 1.09 (0.89) | 1.49 (0.62) | 1.44 (0.59) |
| HR | 2.84 (0.43) | 0.67 (0.54) | 0 (0) | 1.64 (0.67) |
| HM | - | @ | 1.13** (0.92) | 0.98 (0.80) |
| RM | - | @ | 0.97** (0.79) | 0.87 (0.71) |
| HRM | - | @ | 0** (0) | 0.87 (0.71) |

* *At 2 weeks after infection, the mean $log_{10}$ CFU was 4.95*
@ *Same values as for isoniazid (H), rifampicin (R) and HR respectively, since metronidazole was not yet added at these time points.*
** *1 month after start of metronidazole (M). Values in bracket indicate standard error of the mean.*

It was observed that mice treated with metronidazole in combination with rifampicin or rifampicin and isoniazid M.tuberculosis became undetectable in the lungs at the end of 3 months, while they are still present in the lungs of mice treated with metronidazole and isoniazid. Treatment with metronidazole and rifampicin for the duration used in the present study was not able to reduce the M.tuberculosis counts in the spleen to undetectable level even though they were lower than that in the spleen of mice treated with rifampicin alone. A longer duration of treatment withe these combinations may be required in order to sterilise the organs.

Our findings that metronidazole exhibited an additive effect against tubercle bacilli when combined with rifampicin, suggests that metronidazole may have value in eliminating persisters and thus in reducing relapse rates. However, further studies are warranted.

## Multicentric study for diagnostic criteria in childhood tuberculosis

A multicentric study was started in 1995 to develop reliable and objective clinical criteria for the diagnosis of childhood tuberculosis. The centres involved in the study are the Pediatric departments of the Govt. Stanley Medical College, Sri Ramachandra Medical college and the Child's Trust Hospital, Chennai. Children aged6 months to 12 years who had symptoms that warranted investigation for tuberculosis formed the study population.

Ongoing study 1995-1999

**A** total of 2749 children were registered in the study and all examinations at intake work completed in 90%. Symptomatic children were followed up at 2 weeks, 4 weeks, 8 weeks and at 3 monthly intervals upto one year. They were examined clinically and chest X-ray, Mantoux test and gastric lavage for AFB smear and culture were done. Lymph node biopsies were done if indicated. In all, 234 children were found to be bacteriologically or histopathologically positive for TB. So far, 492 children have been started on anti-tuberculous drugs. Among them, the diagnosis was confirmed bacteriologically in 210 and in the rest was based on clinical, histopathological and radiological features.

We have followed 1074 subjects in whom all the clinical and laboratory investigations were done completely upto one year. A random sample of 133 children who defaulted for follow-up were also studied. Clinical and radiographic features of bacteriologically confirmed cases will be compared with those who did not develop active disease upto one year in order to develop diagnostic criteria for TB in children. The data is presently being analyzed.

All children who were diagnosed to have bacteriologically confirmed TB and started on ATT are currently being followed-up to assess their status at the end of treatment. This part of the study is in progress.

## Evaluation of TRC4 for diagnosis of tuberculosis

Agreement between PCR (TRC4) and clinical TBM

| | | PCR(TRC4) | | |
|---|---|---|---|---|
| | | Pos. | Neg. | Total |
| Clinical TBM | Pos. | 61 | 6 | 67 |
| | Neg. | 7 | 22 | 29 |
| | Total | 68 | 28 | 96 |

- Crude agreement = 86%
- Chance corrected agreement = 58%
- Kappa = 0.67

**W**e have evaluated PCR using TRC4 for diagnosis of extrapulmonary tuberculosis during this year.

One hundred lymphnodes (50 fresh and 50 fixed specimens) were used for evaluation of PCR in detecting *M.tuberculosis.* PCR results using pTRC4 and IS6110 were compared with smear, culture and histopathology. With culture and/or histopathology as gold standard, the sensitivity and specificity of PCR using TRC4 primers were 80% and 45%, and PCR using IS6110 primers were 70% and 46% respectively. The specificity of PCR was low. The probes were not significantly different.

Completed study 1996-1998

Similar evaluation was done with 96 CSF samples. Among these 96 samples only 20 were culture positive. TRC4 was positive in all the 20 samples and IS6110 was positive in 19 out of 20 samples. The table gives the PCR results for all the 96 samples.

PCR with TRC4 and IS6110 is still an adjunct for clinical diagnosis. Definitive early diagnosis needs development.

## Rapid drug susceptibility testing of *M.tuberculosis* cultures by luciferace reporter phage (LRP) assay using phAE85 and phAE129

When the first generation TM4 mycobacteriophage, phAE40 was used for drug susceptibility testing of 35 *M.tuberculosis* cultures and the results compared with the conventional method, there was an agreement of 69%, 89%, 83% and 54% for streptomycin, isoniazid, rifampicin and ethambutol respectively. The sensitivity of the assay was poor and the assay required atleast $10^3$ organisms/ml.

The third generation TM4 phage, phAE85, produces 50 times more relative light units and holds promise for improving the sensitivity of the LRP assay. This phage along with the D29 amber mutant, phAE129,was chosen to be used in LRP assay for 70 *M. tuberculosis* cultures of varying sensitivity pattern. This drug susceptibility testing was done for H and R only as the previous assay showed better correlation only with these drugs.

A modified rapid version of the assay, where drug susceptibility results are available within 3 days, is also being tested with both the phages. The above results will be compared and correlated with the results by conventional method and assessed. The study is in progress.

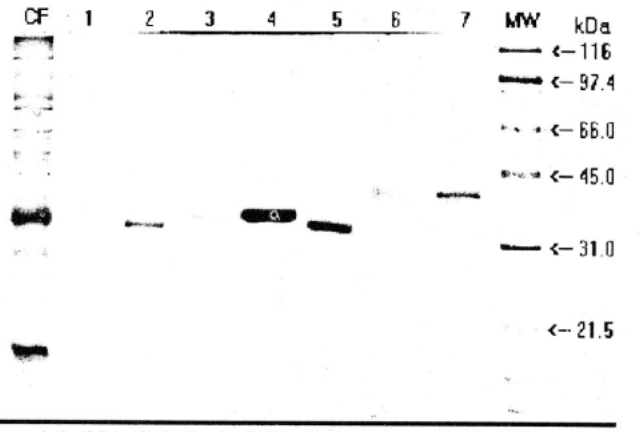
(Ongoing study 1998-99)

## Purification of *M. tuberculosis* antigens and immunodiagnosis

This purification was based on the property of differences in their hydrophobicity, by running on a Phenyl Sepharose HP Column. SDS-PAGE picture of the separated components, along with other reference antigens, is shown in the figure.

Separation of the individual components of the Antigen 85 (Ag 85) complex: Ag 85 complex, isolated by preparatory isoelectric focussing, was subjected to further purification into the individual components namely 85 A, 85 B and 85 C.

CBB stained gel showing the pattern of the purified antigens




Lane 1.Ag85A, 2. 85B, 3. 85C, 4.r85C, 5. Ag6, 6. 38kDa, 7. r38kDa. MW : Standard molecular weight; CF : Culture filtrate

Ongoing study, 1995-2000

Application of the Ag 85 complex and 38kDa antigen for serological testing :

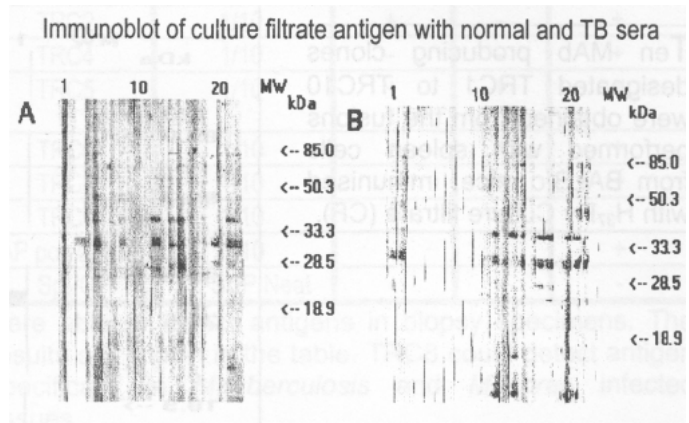
immunoblot: Sera from 20 patients with active pulmonary tuberculosis and 20 normal subjects were tested for qualitative analysis by immunoblot. CF was resolved by SDS-PAGE and transferred to nitrocellulose. The antigen containing strips were treated with 1:100 dilution of sera. The pattern developed is shown in figure.

| Sensitivity and specificity of ELISA with purified antigens | | |
|---|---|---|
| *Antigen* | *Sensitivity (%)* | *Specificity (%)* |
| *$H_{37}Rv$ CF* | 52 | 80 |
| *85A* | 28 | 95 |
| *85B* | 40 | 95 |
| *85C* | 12 | 95 |
| *r85C* | 32 | 95 |
| *38* | 52 | 100 |
| *r38* | 60 | 100 |
| *Ag6* | 60 | 100 |

| Sensitivity and specificity of ELISA by combination of antigens | | |
|---|---|---|
| *Antigen* | *Sensitivity (%)* | *Specificity (%)* |
| *85B &38* | 64 | 95 |
| *Ag6 & 38* | 76 | 100 |
| *85B & r38* | 64 | 95 |
| *Ag6 & 38* | 76 | 100 |
| *38 & r38* | 76 | 100 |

Sixteen out of 20 TB sera recognized the 30/31 kDa (80%), while 5/20 control (25%),sera also recognized the doublet. Recognition of 38 kDa was 75% sensitive (15/20), and 85% specific (3/20). Combination of the result did not increase the sensitivity of the assay.

ELISA: The titres of IgG antibodies in the above sera were determined for their reactivity to the individual components of the Ag 85 complex and to the 38kDa antigen. In addition to the antigens purified at TRC, reference antigens from other sources were also included - such as r38kDa, rAg85C and Ag 6 (30kDa), obtained as gift.




Strips 1 to 10 and 11 to 20 of blots A and B correspond to the reactivity pattern of the control subjects and TB patients to the resolved CF antigens on the nitrocellulose membrane respectively. MW: Molecular weight standards.

At a serum dilution of 1:100, the O.Ds were compared and Mean ± 2 S.D. of the control O.Ds, was considered as the cut-off value. Using this cut-off, the sensitivity and specificity of ELISA with these antigens was calculated and is shown in table.

The 38kDa (purified at TRC) showed a sensitivity of 52%, with 100% specificity. Recombinant 38 kDa and Ag6 were 60% sensitive and 100% specific. Of the 3 components of Ag 85 Complex, the 85B detected more number of positives among the patients. Lesser sensitivity was observed with the antigen 85A, 85C and r85C.

Combination of ELISA results obtained with different antisens: Different combinations of results obtained by ELISA with the 30kDa (85B), Ag6, 38kDa and r38kDa antigens were analysed in order to increase the sensitivity of the assay while keeping the specificity high.

The results of the combination are given in the table. The combination of either the native 38 kDa or recombinant 38 kDa, with Ag6 was offering the maximum sensitivity and specificity of 76% and 100%, respectively.

# Production and characterisation of monoclonal antibodies (MAbs)

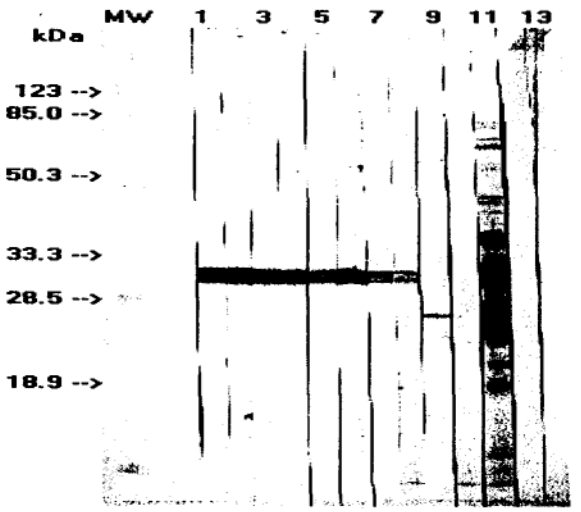
Ten MAb producing clones designated TRC1 to TRC10 were obtained from the fusions performed with spleen cells from BALB/c mice, immunised with $H_{37}Rv$ Culture filtrate (CF).

Completed study 1995-98

The reactivity pattern of ten MAb producing clones with CF has been shown in the figure below:

*Immunoblot analysis of ten monoclonal antibodies with M. tuberculosis $H_{37}Rv$ CF Antigen*

**(Completed study, 1995-98)**




**Lanes 1 - 10** : TRC 1 - TRC 10; **Lane 12 :** Sp2/O TC supernatant ( -ve control)
**Lane 11** : Mouse anti - H37Rv CF sera ( + ve control) **Lane 13** : PBST (blank)

Out of the 10, 7 MAbs -TRC1 to TRC7- reacted with the 30/31kDa doublet(Ag85 Complex); TRC8 with 12kDa in addition to 30/31kDa and TRC9 and TRC10 with the 24 and 12kDa antigens respectively. Table below shows the sub-class of the ten MAbs produced and the molecular weight of the antigens recognised. TRC1 recognised the carbohydrate moiety of the antigen, as evidenced by periodate treatment, while TRC2 to TRC10 recognised the protein epitopes of the antigen.

Based on the reactivity of the MAbs to the atypical mycobacterial species, they were classified as broad (reacting with more than 2 species) or limited cross reactive (with 1 or 2 species) MAbs. TRC8 and 10 were highly specific showing reactivity only with $H_{37}Ra$, South Indian low virulent isolate and not even with *M. bovis.* TRC1 and 2 showed limited cross reactivity with atypical mycobacteria and were also found to react with *E. coli.* TRC3-7 and TRC 9 were catagorised under the broadly cross reactive group. .

Use of MAbs in immuno-assays: An antigen capture assay was developed with hyperimmune rabbit anti-$H_{37}Rv$ CF as "capture" antibody followed by detection with different MAbs. TRC8 was found most suitable and

its lower detection limit corresponded to 156 ng/ml. Using TRC8, the antigen was detected in 17/25 (68%) patients and 3/20 (15%) controls.

The MAbs were also evaluated for their potential to detect mycobacterial antigens from infected tissue, using immunoperoxidase staining.

| Characterisation of MAbs | | |
|---|---|---|
| MAb | Immuno-globulin subclass | Mol. Wt. of reactive antigen (kDa) |
| TRC1 | IgG1 | 30/31 |
| TRC2 | IgG2b | 30/31 |
| TRC3 | IgG1 | 30/31 |
| TRC4 | IgG2b | 30/31 |
| TRC5 | IgG1 | 30/31 |
| TRC6 | IgM | 30/31 |
| TRC7 | IgM | 30/31 |
| TRC8 | IgG1 | 30/31, 12 |
| TRC9 | IgG1 | 24 |
| TRC10 | IgG1 | 12 |

Skin sections of guinea pigs sensitised with *M.tuberculosis* $H_{37}Rv$ were used. Sections from leprosy lesions, as well as lymphnode specimens from Hodgkin's disease were also included. Of the 10 MAbs tested, 6

| Immunohistochemical detection of mycobacterial antigens | | | | |
|---|---|---|---|---|
| MAbs | Dilution | Hodgkins | M.Tb | M.leprae |
| TRC2 | 1/10 | + | - | + |
| TRC4 | 1/10 | - | - | + |
| TRC5 | 1/10 | + | + | + |
| TRC6 | 1/10 | + | + | + |
| TRC8 | 1/10 | - | + | + |
| TRC9 | 1/10 | + | + | - |
| AP polyclonal | 1/10 | - | + | + |
| $Sp_2/O$ | TC SUP Neat | - | - | - |

were able to detect antigens in biopsy specimens. The results are shown in the table. TRC8 could detect antigen specifically in *M.tuberculosis* and *M.leprae* infected tissues.

To summarise, MAb TRC8 was found useful in antigen detection assays and highly specific in reactivity only to *M.tuberculosis.* The fact that TRC8 assay measures the 30/31 kDa antigen, one of the dominant and early secreted antigens adds emphasis to expected value of the test for early diagnosis. TRC8 can be further evaluated in immunoperoxidase staining of infected material, particularly in HIV-TB, where disease due to non-tuberculous mycobacteria, are more common.

## Tuberculosis Facts

- ☹ TB bacilli multiply every 18 hours, compared to every 11-20 minutes for most bacteria.
- ☹ Intermittent treatment is as effective as daily treatment as shown by clinical trials at TRC and elsewhere.
- ☹ Supervised intermittent treatment is more effective than unsupervised daily regimen.
- ☹ Intermittent treatment facilitates Directly Observed Treatment.
- ☹ Intermittent treatment reduces cost.
- ☹ TB is much more common in persons with HIV infection.
- ☹ Thiacetazone should never be given to patients with HIV/TB.
- ☹ Ensure correct sterilisation/disposal of needles if streptomycin is used.

# Study of the cellular immune response to *Mycobacterium tuberculosis* in children

The growing burden of tuberculosis worldwide calls for the development of new vaccines or immunomodulatory approaches, in order to improve the outcome of tuberculosis and if possible, to prevent the development of disease. This depends on an improved understanding of the human immune response to *Mycobacterium tuberculosis.* The clinical outcome of infection with *Mycobacterium tuberculosis* depends on the efficacy of the cell mediated immune response. The chief players in the immune response are macrophages T cells which interact through production of cytokines. It is currently believed that Th1–like cells that produce Interferon-gamma and IL-2 are important in mediating protection against tuberculosis but the role of Th2 cells in enhancing susceptibility to tuberculosis remains uncertain. Several studies done in adults have compared the cell mediated immune response in tuberculosis patients with healthy individuals. These studies have generally found depressed Th1 responses while Th2 responses are not enhanced either systemically or at the site of disease. Some adult studies have also reported lymphopenia, particularly of CD4+ T cells, which reverses with treatment.

. Completed study, 1996-98

The study of tuberculous infection in children provides a unique opportunity to evaluate the initial human immune response to infection and to gain insight into the immunological factors that mediate protective immunity against tuberculosis. Most children infected with *M.tuberculosis* control the infection successfully, remain healthy and become tuberculin positive. Five to 10% develop clinical disease (primary pulmonary tuberculosis) and a small minority suffer severe complications like miliary or disseminated tuberculosis. In order to understand the primary immune response to tuberculosis, we studied cytokine production and lymphocyte subset profile in children with primary TB and compared it to that in healthy tuberculin reactors.

Subjects for this study were drawn from patients attending the pediatric outpatient facility of Government Stanley hospital and Institute of Child Health in Madras city. Two groups of children were studied:

a) Children with tuberculosis were diagnosed by clinical features, persistent.X-ray abnormality, tuberculin test results and appropriate mycobacterial cultures. There were 24 children in this group of whom one had tuberculous meningitis, one had. disseminated TB and the rest had pulmonary tuberculosis. All patients were HIV negative. The mean age was 6.0 ± 0.7 (SEM) years and the mean size of tuberculin reaction was 15.2 ± 1.2 mm. Two patients had negative tuberculin reactions. M.tuberculosis was isolated from sputum or gastric lavage cultures in 16 children and diagnosis was based on clinical grounds in the rest. All patients had received less than 1 week of anti-tuberculosis therapy prior to obtaining blood for this study.
b) Healthy tuberculin positive children who attended the hospital for minor complaints and were found to be free of tuberculosis disease on work up. There were 22 children in this group with a mean age of 6.6 ± 0.8 years and mean size of tuberculin reaction was 16.2 ± 0.7 mm.

The study was approved by the Institutional review boards of the Tuberculosis Research Centre and the Los Angeles County- University of Southern California Medical Center. Informed consent was obtained from the parents before drawing blood.

Preparation of mononuclear cells and stimulation with *M. tuberculosis:* Peripheral blood mononuclear cells (PBMC) were isolated by differential centrifugation over Ficoll-Hypaque and plated in 24 well tissue culture plates at $2x10^6$ cells/ml in RPMI. PBMC were cultured in media alone, with heat killed *M.tuberculosis Erdman* (10 µg/ml), Purified protein derivative 10 µg/ml or with

phytohemagglutinin 10 µg/ml. Supernatants were harvested at different time points and cells and supernatants stored at -70°C till further analysis. ELISA was performed on the supernatants for the cytokines IFN-y, IL-4, IL-10 and IL-12. Total cellular RNA was extracted from cells and cDNA synthesised. Competitive PCR was performed to evaluate the mRNA signal for IFN-y production after normalising samples for CD3 cDNA content.

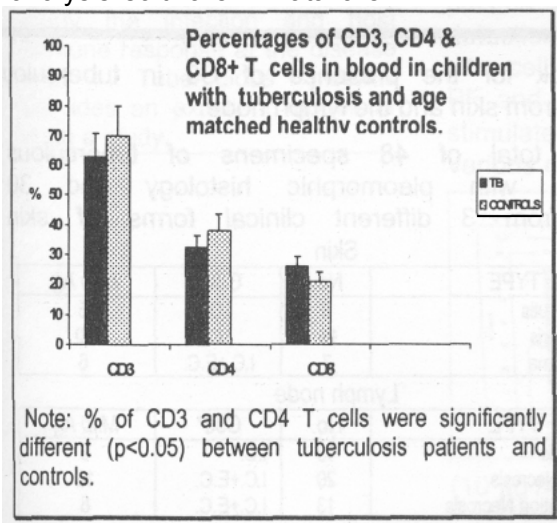
One aliqot of cells was used for measuring lymphocyte subsets. About 200,000 cells were added to each tube of a panel of six monoclonal antibodies and processed. Cell subsets were determined FACS using a BD flow cytometer and Cell Quest analysis software. Data are expressed as Mean ± SEM (Standard error of the mean).

Interferon-gamma production by *M. tuberculosis* - stimulated PBMC: Table shows the production of different cytokines by PBMC when stimulated with tuberculosis antigens and a nonspecific mitogen, PHA. There was an antigen specific reduction in Interferon-gamma production in patients with tuberculosis but no difference in IL-10 or IL-12 production between the two groups. IL-4 was not detectable in the antigen stimulated cells in either group.

Correlation of clinical status and IFN-y production: Among patients with tuberculosis, we found wide variation in the production of IFN-y. In PPD stimulated PBMC, the mean interferon-gamma concentration in 17 children with moderate or far advanced disease was 215 ± 14 µg/ml compared to 534 ± 59 µg/ml in 7 children with minimal parenchymal lesions(p=0.02). IFN-y production was also reduced in 10 malnourished patients, compared to 14 paients who were not malnourished ( 175 ± 23 vs 407 ± 26 µg/ml, p = 0.03).

Production of IFN-γ, IL-10 and IL-12 in tuberculosis patients and controls

| | IFN-γ (pg/ml) | | IL-10 (pg/ml) | IL-12 (pg/ml) |
|---|---|---|---|---|
| | *M.tb* antigen | PHA | *M.tb* antigen | *M.tb* antigen |
| Tuberculosis | 465 ± 138 | 1634 ± 351 | 1476 ± 586 | 316 ± 87 |
| Control | 1363 ± 323 | 1552 ± 295 | 881 ± 265 | 260 ± 118 |
| *p-value* | *0.02* | *NS* | *NS* | *NS* |



Note: % of CD3 and CD4 T cells were significantly different (p<0.05) between tuberculosis patients and controls.

Lymphocyte subset profile. Figure shows the percentages of CD3+, CD4+, and CD8+ T cells in patients and controls. The percentage of CD3+ (63 ± 2.6 vs 70 ± 1.7%, p<0.03) and CD4+ cells (32.5 ± 2.0 vs 38 ± 2.2 %, p< 0.03) in the blood was significantly lower in tuberculosis patients. The CD4/CD8 ratio was also lower in tuberculosis patients compared to controls (1.4 ± 0.13 vs 1:9 ± 0.19, p=0.02). There were no differences in the percentages of Pan TCR$\gamma\delta$, V$\delta$1 and V$\delta$2 subtypes of lymphocytes between the two groups. When absolute cell numbers were calculated, the total lymphocyte count (3173 ± 569 vs 5413 ± 361 cells/cu.mm, p=0.006) and CD4+ T cell count (971 + 180 vs 2223 + 319 cells/ cu.mm, p=0.002) were significantly lower in tuberculosis patients compare to controls. Eight patients could be studied after treatment as well. Though the percentage of cd3+ cells had increased post-treatment, it was not statistically significantly different from pre-treatment levels, probaly because of the wide variation in pre-treatment levels. However, there was a significant increase in the proportion of CD4+ T cells in blood following chemotherapy (34±1.8 vs 27.4±3.7, p<0.04), indicating that tuberculosis produces a reversible suppression of systemic T helper cells.

Deficiencies in both the qualitative and quantitative aspects of the immune response have been shown to have an impact on clinical outcome in patients infected with *M. tuberculosis.* The cellular immune response against *M.tb* involves activation of the Th1 subset of CD4+ T cells with production of cytokines such as IL-2 and IFN-γ. Several studies in adults have found a depressed Th1 response in active TB and there also appears to be a correlation between severity of disease and extent of immune suppression[1]. There have also been reports, mainly in adults, that active TB is associated with lymphopenia particularly CD4+ lymphopenia. This appears to be a reversible phenomenon with lymphocyte counts increasing following anti-TB treatment.

Our findings demonstrate that, during the initial immune response to *M.tuberculosis* in children, tuberculosis antigen- induced IFN-γ production by PBMC correlates strongly with clinical outcome. This was lowest in patients with more severe disease. However this depressed Th1 response was not associated with an enhanced Th2 response. We also found that the percentage and absolute numbers of total lymphocytes, CD3+ and CD4+ T cells in blood was reduced in children with tuberculosis. The CD4/CD8 ratio was also decreased. However, the percentage of Pan TCRγδ, Vdelta1 and Vdelta 2 subsets was not different in the 2 groups of children. In a subset of children who could be examined again, the percentage of CD4+ T cells had increased significantly following treatment; though the percentage of CD3+ T cells also increased, it did not reach a level of significance.

It appears that in addition to a qualitative defect in the production of Th1 cytokines, tuberculosis is also associated with a systemic decrease in the absolute numbers of CD3+ and CD4+ T cells. Whether CD4+ lymphocytopenia is a cause or effect of the severity of disease is unknown. The mechanism of low CD4+ counts in TB is also unknown. Additional studies are required to delineate the mechanisms underlying the systemic lymphopenia and depressed Th1 response in human tuberculosis.

## **Complement in the pathogenesis of necrosis in** tuberculous **granuloma**

Activation of complement by mycobacteria, presence of activated complement products in patients' sera and complement-mediated phagocytosis of mycobacteria are known. It is therefore believed that complement may play an important role in the formation of tuberculous granuloma.

Aim: To look for the presence of C3 in tuberculous granulomata from skin and the lymph node.

Method: A total of 48 specimens of tuberculous lymphadenitis with plemorphic histology and 30 specimens from 3 different clinical forms of skin tuberculosis were stained with anti-C3 or *M.tuberculosis* antigen using appropriate antibody and the indirect immunoperoxidase method.

Skin

| TYPE | No. | C3d* | *M.tb* Ag |
|---|---|---|---|
| Verrucosa cutis | 8 | I.C. | 5 |
| Lupus vulgaris | 15 | I.C. | 10 |
| Scrofuloderma | 7 | I.C.+E.C. | 6 |

Lymph node

| TYPE | No. | C3d* | *M.tb* Ag |
|---|---|---|---|
| Non-Necrotic | 15 | I.C. | 9 |
| Caseation Necrosis | 20 | I.C.+E.C. | 12 |
| Non-Caseation Necrosis | 13 | I.C.+E.C. | 8 |

* Complement C3 present in all specimens either intracellularly (IC) alone or both intracellularly and extracellularly (I.C + E.C)

Complement staining seen in all specimens examined; seen intra cellularly in non-necrotic granulomata; present additionally in areas of necrosis; B cells, plasma cells and mycobacterial antigen(s) observed more frequently in necrotic granulomata.

Completed study, 1998

# Immune response in tuberculous pleuritis: A comparison of local vs. systemic responses

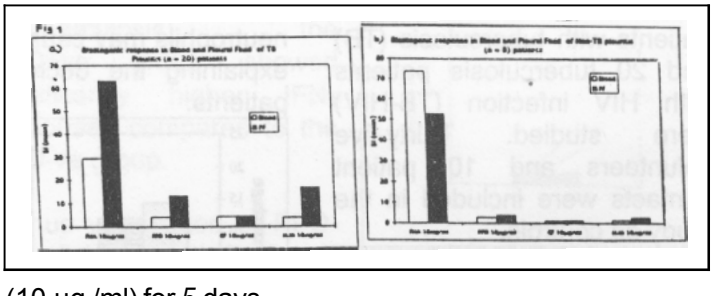
Among the many clinical manifestations of tuberculosis, pleuritis is of particular interest since it resolves without chemotherapy and the patients are known to mount a relatively effective immune response *against M.tuberculosis* infection. Immunity against tuberculosis is primary cell mediated which seems to be compartmentalised into local and systemic, the local response being more intense. It would, therefore be worthwhile to study the infection and host immune response at the disease site. Tuberculous pleuritis provides an excellent model for such a study.

Ongoing study, 1998-2003

The planning of the project was done in the first quarter of the year. The focus of the project was to look into the cell mediated immune responses and draw a comparison between the local and the systemic infection. So far 24 pleuritis (PF and Blood) samples were collected and processed. Initial diagnosis was drawn from clinical history. PF samples were sent to bacteriology for culture and smear. All were negative by smear and only 2 out of 20 were positive for TB pleuritis by culture.

Biochemical assays were done for total protein and glucose on all the above PF and blood plasma samples. An Adenosine Deaminase Assay was also set up with the above samples. The samples were also stored at -20°C for PCR based diagnosis. This method of diagnosis is based on the IS6110 repetitive element in the mycobacterial genome. All non-TB pleurisy PF and blood samples were included in the control group.

In vitro cytokine assays were set up by stimulating PF and the blood cells of patients with mitogen (PHA 1 µg /ml or 10 µg /ml) and with various mycobacterial antignes (PPD, CF, HK M.tb at 10 µg/ml). The cells were harvested at varying time points of 24 h, 48 h and 72 h, lysed with RNA Zol and stored at -20°C for RT-PCR. The supernatants of these were stored for quantitative cytokine assay ELISA. PF supernatants and blood plasma were also stored at -20°C for antigen antibody detection assay by Western blot and ELISA. In vitro cell proliferation assays (LTT) were performed for all PF and blood samples. Cells in each experiment were stimulated with mitogen (PHA 1µg/ml) for 3 days and with various mycobacterial antigens viz. PPD, CF and HK M.tb



(10 µg /ml) for 5 days.

LTT results (Fig.1a & b) indicate an increase in cell proliferation in both blood and PF when stimulated with PHA. In most subjects with TB Pleuritis there is an enhanced reactivity to mycobacterial antigens which is manifested by accelerated cell proliferation kinetics. In most of these cases the Stimulation Index (SI) in PF in response to these antigens seems to be more pronounced. When a comparison was drawn with control PF and blood (non-TB Pleuritis), it was seen that though there was a very high stimulation to PHA not all of these controls showed a significant response to mycobacterial antigens.

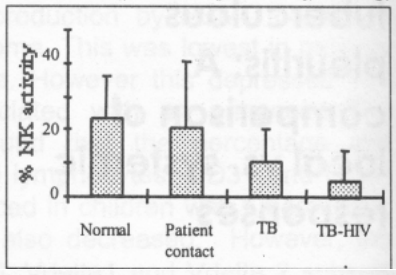
# Role of natural killer cells in tuberculosis

Human infection with *Mycobacterium tuberculosis* displays a spectrum of manifestations that reflect the efficacy of the immune response. In the existent literature the number of works devoted to the sub-population of NK cells is not significant. Hence the purpose of the present study was to examine how immune parameters related to non-major histocompatibility restricted cytotoxicity changed with respect to the presence of tuberculosis. HIV infected individuals are subjected to various immunity disorders resulting in severe opportunistic infections. When HIV infection is associated with tuberculosis there is a more accelerated immune deterioration. Therefore, 35 patients with tuberculosis (TB) and 20 tuberculosis patients with HIV infection (TB-HIV) were studied. Thirty-five volunteers and 10 patient contacts were included in the study as controls.

Ongoing study, 1997-98

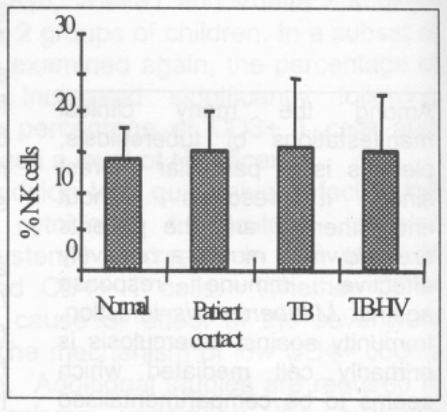
Phenotyping:

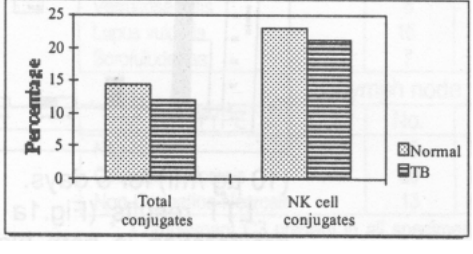
Phenotyping of PBMC was done by flow cytometry using CD3/CD16+56 monoclonal antibodies, tagged to fluorochromes FITC/PE respectively. No changes were observed between the different groups with respect to the number of NK cells.




Cytotoxicity:

Chromium labeled U937 cells of myeloid lineage were used as tumor targets and PBMC from various groups were used as effectors at a target: effector ratio of 50:1. Per-centage killing is directly proportional to the counts of chromium obtained in the supernatant. There was a significant decrease in NK activity in patients as compared to controls, whereas there was no difference between the contact and patient groups. *In vitro* experiments have shown that cellular regulation of NK cells by monocytes/neutrophils is mediated through $H_2O_2$ production. In *M. tuberculosis* infection, it has been proved *in vitro* that reactive oxygen intermediate (ROI) levels are increased. This increased ROI by monocytes or neutrophils may cause suppression of NK activity possibly explaining the decreased activity seen in tuberculosis patients.




Conjugate study; In order to understand the defect in killing, one must try to delineate at which step there is a defect: whether it is at the recognition stage or binding stage or at the death signal level. Attempts were therefore made to see whether there was a defect in the binding mechanism in the TB patients. It appears that the NK cells in TB patients are as efficient in recognizing their target cells as the NK cells in the normal population. The mechanism of defect among tuberculosis patients in the cytotoxic effect therefore is not at the binding stage but probably lies in the subsequent events involved in the lethal hit.

# Cytokine profiles in pre and post BCG vaccinated adult population - Analysis by PCR detection of cytokine mRNA and ELISA

In continuation of the Annual Report of 1996-97, the data obtained by RT-PCR and ELISA were analysed. Tuberculin skin-test results (Table 1) showed alterations in the T cell repertoire. However, no relationship between DTH to tuberculin and protection was established. In vitro proliferative responses to PHA, PPD and *M.tuberculosis* remained largely unaltered by vaccination.

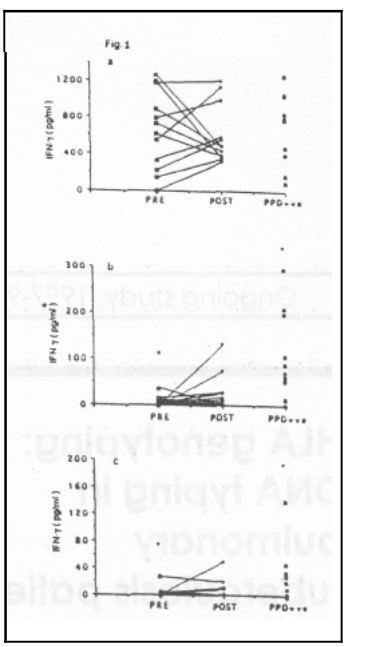
Skin test responses to tuberculin (1 TU RT 23) before (PRE) and 8 weeks after (POST) BCG vaccination.

| No.of | PRE | POST | PPD +VE |
|---|---|---|---|
| subjects | 17 | 17 | 17 |
| Age range (Mean) | 13-22 (16.5) | 13-22 (16.5) | 13-25 (18.6) |
| Sex | 11M/6F | 11M/6F | 10M/7F |
| BCG scar range (Mean) | 0 | 6-9mm (7.5mm) | Not seen |
| PPD reaction size (Mean±S.E.) | 4.58mm±1.32 | 14mm±4.1 | 21.12mm±3.2 |

Cytokine response by RT-PCR & ELISA: Cytokine profile studied by RT-PCR was semi-quantitative. In pre (PPD-ve) group 75-80% expressed IFNg, IL4 and IL10 by PHA stimulation while 40-60% following PPD and *M.tuberculosis* stimulation. The expression levels did not changed after BCG vaccination. In PPD+ve group 40-60% expressed all three cytokines following PHA stimulation and 15-50% following PPD or *M.tuberculosis* stimulation.

We know that BCG vaccination has not given protection against tuberculosis in South India, however, it causes skin conversion from a mantoux negative to a positive status. This shows that BCG cause certain alterations in the T cell repertoire which in might alter the cytokine secretion pattern. Hence, there is a need for conducting a phenotypic functional analysis of T cells in PRE and POST BCG vaccinated subjects to assess whether vaccination modulates the immune response towards protection in this population.

The quantitative data by ELSA (Fig.1) showed no difference in production of IFNγ between the three groups when stimulated with PHA. With mycobacterial antigens (PPD & *M.tuberculosis),* the PPD+ve group showed significantly higher IFNγg responses compared to the PPD-ve group.




Thus vaccination of PPD-ve individuals with BCG had little effect on IFNγ production. This suggests that firstly, unvaccinated PPD+ve individuals in this area have developed a TH1 type of response. to mycobacteria possibly due to their exposure to *M.tuberculosis* or environmental mycobacteria.

The finding also suggests that BCG has had little effect in driving the immune response towards a protective TH1 type in this population. Our results on IL4 and IL10 levels indicate that there is no significant change induced by BCG vaccination.

Completed study, 1993-98

## HLA-DR2 phenotype and plasma lysozyme in pulmonary tuberculosis

The main aim of this present study is to understand the role of HLA-DR2 and non-DR2 genes/gene products on the mechanism of non-specific immunity associated with the susceptibility to tuberculosis.

Preliminary study revealed that plasma lysozyme binds with live *M.tuberculosis.* Initial experiments were carried out with three different doses (i.e. 0.5 mg, 1 mg and 2 mg per 100 µl of plasma) of either heat killed *M.tuberculosis,* live *M.tuberculosis* or heat killed *Micrococcus lysodicticus.* The plasma lysozyme level was measured before and after incubation at different time points (0 hr, 2 h, 4 h and 24 h). The study revealed that maximum binding of lysozyme was achieved at 2 mg/l00 µl of plasma. 4 h and 24 h time points were selected for further studies.

Our earlier study revealed that HLA-DR2 is associated with a decreased level of lysozyme in the plasma of HLA-DR2 positive active pulmonary tuberculosis patients when compared to DR2 negative patients.

The binding activity of plasma lysozyme on live *M.tuberculosis* was studied by incubating *M.tuberculosis* in 100 µl of either active-TB plasma samples (n=14) (DR2 positive n=7 and DR2 negative n=7) or healthy control plasma samples (n=14) (DR2 positive n=7 and DR2 negative n=7). The binding activity of lysozyme at 2 mg/l00 µl concentration at 4 h and 24 h time points were studied. The stimulatory or suppressive activity of the live bacilli treated plasma samples were tested using lymphocyte transformation test. The results are being analysed. Further study on the viability will be carried out next year.

Ongoing study, 1997-99

The study will be useful to understand the influence of HLA-DR2 genes/gene products on the non-specific immune mechanisms involved in the susceptibility/resistance to tuberculosis. The study is progressing.

## HLA genotyping: DNA typing in pulmonary tuberculosis patients and control subjects

Our earlier study revealed an increased phenotype frequency of HLA-DR2 antigen in pulmonary-TB patients ($p<0.00l$). To find out the sub-types of DR2 antigen at the DNA level, genotyping has been planned.

HLA-DR2 positive subjects, (patients and controls) DNA samples will be subjected to Polymerase Chain Reaction using specific primers. HLA-DR2 genotyping will be carried out by dot-blot technique using specific oligonucleotide probes. The study will be carried out in HLA-DR2 positive (n=50) patients and control subjects (n=50). The study has been started and is progressing.

Ongoing study, 1997-99

The present study will be useful to find out the sub-type(s) of HLA-DR2 that is/are associated with the susceptibility to tuberculosis and the mechanism of disease susceptibility.

# Studies on HLA and Non-HLA gene polymorphism in tuberculosis spine (Extra-pulmonary tuberculosis

Studies on HLA-antigen profile in pulmonary tuberulosis revealed a significant increase of HLA-DR2 in PTB patients. Moreover, our recent study on non-HLA gene polymophisms revealed that functional mutant homozygotes of mannose binding protein gene is associated with the susceptibility to pulmonary tuberculosis.

Ongoing study, 1997-99

To find out whether HLA-DR2 or any other antigen (both HLA-Class I and Class II antigens) is/are associated with extrapulmonary forms of tuberculosis, a study on HLA and spinal tuberculosis has been initiated.

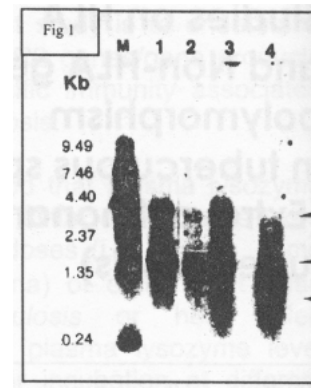
During the year, serological determination of HLA-B, -B,- DR and -DQ has been carried out in 50 patients and 30 family contacts (spouses) of the patients. Further, non-HLA gene polymorphism such as interleukin-1 Receptor Antagonist gene and Vitamin-D Receptor gene was also carried out in these patients and control subjects. Tumor necrosis factor alpha and beta gene polymorphism and mannose binding protein gene polymorphism will be studied in subsequent year.

The study will be carried out in 50 to 75 TB-spine patients and 50 to 75 control subjects consisting of spouses (family contacts) of TB-spine and PTB patients.

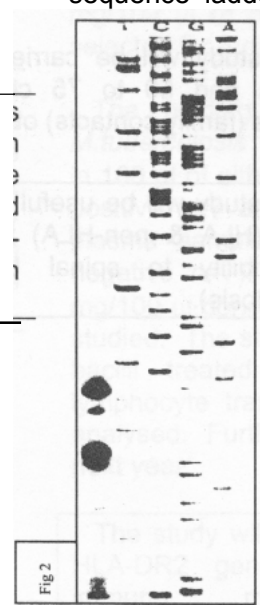
The study will be useful to find out the multicandidate genes (HLA & non-HLA) that are associated with the susceptibility to spinal tuberculosis (Extrapulmonary tuberculosis).

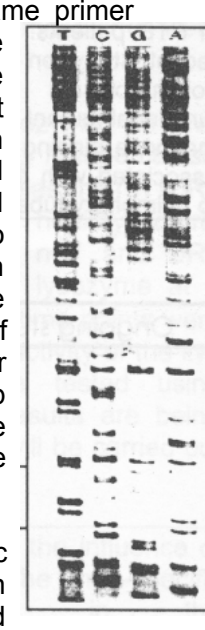
# Identification of the promoter of amidase gene for expression of useful mycobacterial genes

As mentioned in the previous years report, the transcription start points of the amidase promoter has been deduced by northern blot analysis, RT-PCR and primer extension analysis.




Primer extension (PEA) reaction with RT2 primer revealed 2 major products 51 bp and 59 bp and a minor product at 92 bp from the ATG initiation codon of the amidase gene (Fig.1). The other primer extension product was obtained (1863 bp) from the ATG initiation codon. This site was confirmed by using two reverse primers 1220C and also 1359C. Fig.2 shows the primer extension product obtained with 1220C with the sequence ladder obtained with the same primer




Subcloning of the fragments around the PEA products detect promoter activity in E.COLI. We subcloned the fragments around the PEA products to see whether the region just upstream to the coding region of amidase is a promoter or the region 1863 bp upstream of the initiation codon is the promoter.

To find the specific promoter region within 800 bp of AM12 and AM14 fragments which include the PEA products, PCR2.1 vector harboring these fragments were digested with EcoRI, klenow filled and ligated to ScaI digested pJEM13 which is a lacZ promoter probe vector. Subcloning of the Ami II fragment in pJEM13 which includes the PE product around 1863 bp upstream of the coding region of amidase resulted in blue colonies on LB-Kan X-gal plates indicating the expression of lacZ gene whereas AMIIV fragment composing the PE products from 57-92 bp did not give any blue colonies. The orientation of the clones have been checked and confirmed. This indicates that the region 1863 bp upstream of initiation codon ATG is the functional promoter and the region 51-92 bp just upstream is not a promoter and could be the processing site.

This inducible promoter is being used to express other mycobacterial genes useful for diagnosis and protection.

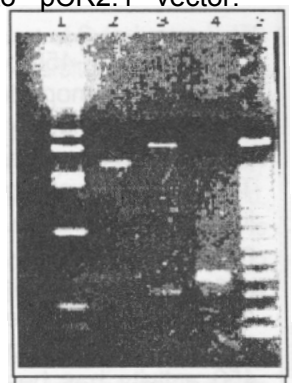
Ongoing study, 1996-2000

# Mycobacterial promoters - a molecular study

Information on gene regulation in mycobacteria is limited. Studies on mycobacterial gene regulation will provide insight into the mechanisms governing pathogenicity and virulence of this slow-growing bacterium.

Enzymes involved in the de *novo* purine biosynthesis are important for infectivity, growth and virulence of certain pathogenic bacteria. Studying the *guaA* gene encoding GMP synthetase from *M. tuberculosis* will help us understand its role in pathogenicity.

**S**alient Findings:The promoter clones isolated during the early phase of this study were sequenced and the transcription start points were determined using primer extension analysis. Homology studies revealed interesting features. One of the promoters exhibited very high homology to the putative *guaA* gene encoding GMP synthetase from *M. tuberculosis.* A 1.6 kb coding region along with 400bp of the upstream region was PCR amplified using specific primers and cloned into pCR2.1 vector.



Gel photograph showing the PCR products obtained using different primer sets with cosmid MTCY78 DNA.
Lanes
←BstEII digest of lambda DNA
↑PCR amplified guaA gene(1.6 kb)
→PCR amplified guaA gene with 400bp upstream sequence (2 kb)
↓PCR amplified 400bp upstream sequence Lane 5 - 100bo ladder

Sequence analysis and restriction mapping confirmed the identity of the cloned fragment to be the *M. tuberculosis guaA* gene. The upstream region of this gene was cloned separately into a promoter-probe vector and studies are underway to determine the transcription start point of this promoter and to study its regulation. The coding region of the gene was cloned into an expression vector and transformed into *E. coli* for overproduction of the gene product. Our results are in line with the earlier observation by other workers that expression of this gene is lethal to *E. coli.* Hence, attempts are being made to express this gene under the control of heat shock promoter (hsp60) in *M. smegmatis* using a shuttle plasmid vector.

The promoter clones isolated in the initial part of this study were further characterised king primer extension analysis.

Experiments are in progress to study the ability of these promoters to drive a candidate mycobacterial gene. we chose the immunodominant 38kDa protein for this purpose A 1.1kb coding region of the 38kDa protein antigen gene was PCR amplified using specific primers and the product was cloned into pCR2.1 vector. This gene will be used to study the transcriptional strengths of the isolated promoters.

-35
5' - GATCATTCGG AGCAG**CCGACT**TGTCAGTGG CTGTCTCTAG
-10 ↓
TGTCACGGCCAACCGCGATACCGGTCAATCGAACACCGAC
RBS
CACAGGAGAG GCACCATGAC GCAGACCCCC - 3'

Primer extension analysis of a cloned promoter depicting the consensus -10 and -35 region (in boldface) with respect to the experimentally determined transcription start site (tss) indicated by an arrow. The probable ribosome binding site (RBS) and the predicted start codon (ATG) are also shown in the figure.

Future Studies: To express the guaA gene in a mycobacterial host and study its functional activity. To determine the transcription start site of this promoter and study the mechanism of its regulation.

Ongoing study, 1997-99

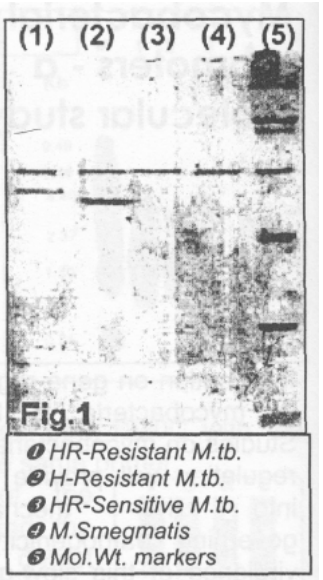
# Microsomal mixed function oxidases in experimental tuberculosis

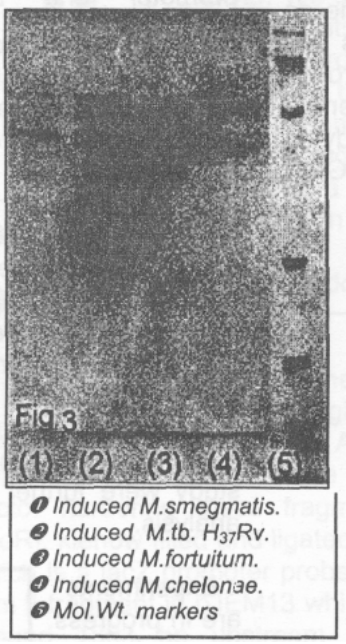
Cytochrome P-450 belongs to a group of hemoproteins which are vital components of the mixed function oxidasesystem. This family of proteins is involved in the oxidative metabolism of a variety of xenoDioiics ana drugs. An association between drug resistance and Cytochrome P-450 activity has been reported in certain living species. Since no detailed studies of Cytochrome P-450 in mycobacteria are available the following experiments were carried out.

Completed study, 1994-98

**A**ims:

1. To purify Cytochrome P-450 in certain mycobacterial species such as *M.smegmatis, Mfortuiturn, M.chelonae* and *M.tuberculosis* $H_{37}R_v$ and certain drug resistant *M.tuberculosis.*
2. To study the inducing effect of phenobarbital on Cytochrome P-450 present in M*.smegmatis, M.fortuitum, M.chelonae* and M.*tuberculosis* $H_{37}R_v$ and purify the induced protein in the above mentioned bacteria.
3. To study the *in vitro* and *in vivo* effects of rifampicin and isoniazid on Cytochrome P-450 in mycobacteria.
4. To study P-450-mediated lipid peroxidation *in vitro* and *in vivo* in the presence of rifampicin, isoniazid, pyrazinamide and ethambutol and compare the findings between drug sensitive and resistant bacteria.

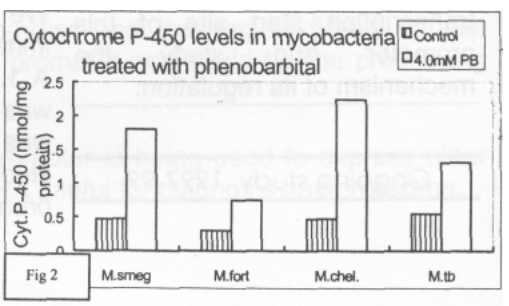



**Findings:**

1. A simple method for purification of Cytochrome P-450 in mycobacteria was developed. Upon purification, the hemoprotein gave a single band corresponding to a molecular weight of 66 kDa in *M.smegmatis, M.fortuitum, M.chelonae* and *M.tuberculosis* $H_{37}R_v$. while that purified from isoniazid resistant and isoniazid and rifampicin-resistant *M.tuberculosis* gave an additional band, corresponding to a molecular wt. of approximately 50-55 kDa, in addition to the single band obtained at 66 kDa(Fig.1).

2. At a concentration of 4.0 mM, phenobar-



bital was able to significantly induce Cytochrome P-450 in *M.smegmatis, M.fortuitum, M.chelonae* and *M.tuberculosis* $H_{37}R_V$ (Fig.2). When purified, the Cytochrome P-450 from the phenobarbital- induced mycobacteria gave an electrophoretic pattern similar to that obtained with the drug resistant *M.tuberculosis* (Fig.3).

3. Rifampicin was able to significantly induce Cytochrome P-450 in *M.smegmatis* and *M.tuberculosis* $H_{37}R_V$ when studied *in vitro,* while isoniazid had an opposite effect. The inhibition of CytochromeP-450 by isoniazid was more pronounced in *M.tuberculosis* $H_{37}$Rv than in *M.smegmatis. In vivo* experiments in rats also gave similar results with rifampicnandisoniazid.

4. Lipid peroxidation was high in the presence of isoniazid and pyrazinamide, followed by rifampicin and was least with ethambutol. The above reaction occurred at an enhanced rate when drug-resistant M.tubercul0si.s homogenate was used as the source for Cytochrome P-450. In vivo results were also similar.

CONCLUSIONS

1. Cytochrome P-450 seems to appear as another isoform in the drug-resistant mycobacteria.
2. It is interesting to note that the same isoform of Cytochrome P-450 exists in the drug-resistant and phenobarbital-induced myco-bacteria.
3. When rifampicin is given along with drugs which are metabolised through the P-450 pathway, the bioavailability of such drugs may be lowered thereby leading to drug resistance. On the other hand, since isoniazid inhibits mycobacterial P-450 activity, this leads to sustained high blood levels of the accompanying drugs due to retarded metabolism. Therefore, chances of toxicity of such drugs is likely to occur.
4. Lipid peroxidation increases with increased Cytochrome P-450, since it has already been shown that the drug-resistant bacteria had elevated P-450 activity. Also, combining isoniazid with pyrazinamide could lead to enhanced lipid peroxidation which could be toxic.

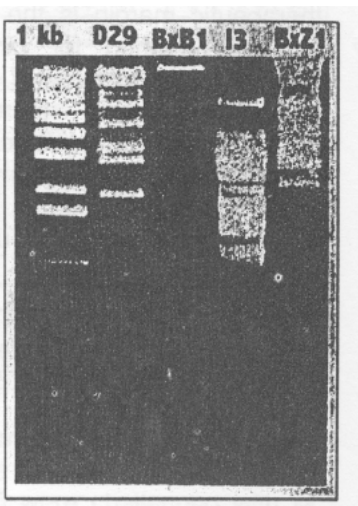
## Isolation of a new mycobacteriophage BXZl

Lysogenic mycobacteriophage infecting *M.tuberculosis* can be of much use in increasing the sensitivity of luciferase reporter phage assay, as the cells are not killed upon infection with the phage and so the availability of ATP is sustained.
Thus, screening environmental samples for new lysogenic mycobacteriophages is one of the approaches to increase the sensitivity of luciferase reporter phage assay.

completed study, 1998

**A** phage termed BXZl, was isolated from soil samples from NewYork. Plaque morphology of this new phage is different from other well known phages.

Based on plaquing pattern on *M. smegmatis* $mc^2$ 155 mutants, the mycobacterio phages can be typed. About 21 $mc^2$ 155 mutants were used to study the plaquing patterns of BXZl and compared with others. Based on this result BXZl can be grouped broadly with 13 and BXBI but well differentiated from them when further subtyped.




Differentiation at molecular level was done by restriction digestion with enzymes Aat11 and CSP45 and gel electrophoresis yielded unique bands with BXZl compared to D29, 13 and BXBI. Complete sequencing of BXZl phage DNA and further characterisation are in progress with the collaborative laboratory.

# A multi-centre study of the early bactericidal activity (EBA) of anti-tuberculosis drugs

The early bactericidal activity (EBA) of anti-tuberculosis drugs has been measured as the fall in colony forming units (CFU) of *M. tuberculosis* in sputum during the start of treatment. The standard EBA is defined as the fall in log CFU counts per ml sputum per day over first 2 days. The ratio between the dose usually used in treatment and the dose just capable of yielding a measurable EBA could be estimated as the therapeutic margin. Estimation of therapeutic margin is the only available method for directly measuring the activity of drug in patients and therefore provides unique information concerning dosage of currently available anti-TB drugs and also of drugs under development.

Completed study, 1998

In 1993, at the suggestion of the WHO Steering Committee on treatment of Mycobacterial diseases (THEMYC), a multi-centre study of the EBA was started. Four collaborating centres situated at Nairobi, Hong Kong, South Africa and TRC, Chennai used the same protocol and central coordination was provided by D.A. Mitchison at St. Georges Hospital Medical School, London.

The aims of the study were (1) to compare estimates of the EBAs obtained by viable counting with those obtained by counting total numbers of acid fast bacilli (AFB), using drugs and dosages with a wide expected range of EBAs; (2) to see whether estimates of the EBAs obtained by total counting could be used to correct and make more precise the EBAs obtained by viable counting; (3) to see whether the EBAs were similar in the different collaborating centres; (4) to see whether extending the treatment period from 2 days to 5 days gave additional information and (5) to compare whether the variation between patients in the participating centres would affect the precise estimation of CFU.

**Mean viable count (log10 CFU/ml) change in counts**

| Regimen | No.of Pts. | S1 | S3 | S6 | S1-S3 | S3-S6 |
|---|---|---|---|---|---|---|
| No drug | 16 | 7.62 | 7.50 | 7.05 | 0.12 | 0.45 |
| INH18.75 | 15 | 7.67 | 7.31 | 6.86 | 0.36 | 0.45 |
| INH300 | 13 | 7.81 | 6.33 | 5.93 | 1.48 | 0.40 |
| Rif. 600 | 13 | 7.67 | 7.26 | 6.53 | 0.41 | 0.73 |
| Ofl. 800 | 13 | 7.15 | 7.39 | 6.94 | -0.24 | 0.45 |

Mean smear count/ml sputum change in counts

| Regimen | No.of Pts. | S1 | S3 | S6 | S1-S3 | S3-S6 |
|---|---|---|---|---|---|---|
| No drug | 16 | 7.30 | 7.19 | 6.97 | 0.11 | 0.22 |
| INH18.75 | 15 | 7.08 | 7.05 | 6.86 | 0.03 | 0.19 |
| INH300 | 13 | 7.27 | 6.43 | 5.60 | 0.84 | 0.83 |
| Rif. 600 | 13 | 7.22 | 7.34 | 6.80 | -0.12 | 0.54 |
| Ofl. 800 | 13 | 7.06 | 7.00 | 6.73 | 0.06 | 0.27 |

Anti-tuberculosis drugs: Patients were randomly allocated by the use of treatment slips inside envelopes, to daily dosage with either isoniazid 300mg (6mg/kg), isoniazid 18.5mg (0.35mg/kg), rifampicin 600mg (12mg/kg) oflaxacin 800mg (16mg/kg) or no drug. These treatment groups are termed INH300, INH18.5, RMP600, OFL800 or Nil. The drug dosages used cover a wide range expected EBA values.

The EBA was measured by counting colony forming units (CFU) and total AFB in sputum collections, taken pretreatment (S1) at 2 days (S3) and at 5 days (S6).

The change in mean viable count (log 10 CFU) and mean smear count pertain to TRC, Chennai patients are as detailed in the table.

The intake to this study in all 4 centres has been completed and results are being analysed by the coordinating centre. Our preliminary analysis reveals that isoniazid produced a massive kill, perhaps on actively growing organisms during the first 2 days but was almost inactive thereafter, whereas rifampicin maintained a moderate activity against slowly growing organisms throughout the period. This finding suggest that EBA measured during the 3-5 day interval might be able to assess the sterilising activity of the drug. Oflaxacin had moderately high mean S1-S3. A good correlation between the initial S1 CFU and total count was also found in this study.

# Protective effect of 65 kDa HSB vaccine against *M.tuberculosis* infection in the guinea pig model

DNA vaccines are newer addition to the potential vaccine technologies and offer promise for the improvement of existing vaccines. DNA vaccines consist of plasmid DNA expression vectors that, when administered to an animal, result in expression of an antigen *in situ* leading antigen specific immunity. Lowrie *et al* have shown that mice injected intramuscularly with mycobacterial DNA encoding the hsp 65 antigen were protected against challenge with *M. tuberculosis* infection and the protection observed was greater than that offered by BCG.

Completed study, 1997-98

**S**ince guinea pig model for experimental tuberculosis exhibits a remarkable similarity to that of tuberculosis observed in human both in terms of granuloma formation and hypersensitivity responses, we have used this model to test the protective effect of 65 KDa HSP vaccine against *M. tuberculosis* infection. The details of experiments carried out are as follows:

Immunisation: A total of 72 guinea pigs were randomly allocated in to 6 groups of each 12. The guinea pigs in Groups 1 & 2 (control group) were immunised by intramuscular injection with 180µg (45x4) of vector DNA. The guinea pigs in Group 3 & 4 were immunised by intramuscular injection with 1 mg of BCG in 0.1ml distilled water prepared from a 2 week old growth on L.J. The guinea pigs in group 5 & 6 were intramuscularly injected with 200 to 240 µg of hsp65 DNA in 0.1ml, in 4 different sites, at 3 time points with 2 weeks intervals between each doses, thereby each animal received 680µg of hsp65 DNA.

Skin test: Four guinea pigs selected at random from BCG groups were skin tested intradermally using 5 TU of PPD-S in 0.1ml and were read on days 1, 3, 5, 7, 14 & 21. Four guinea pigs from hsp65 DNA groups were skin tested intradermally with 250µg of a single dose of hsp65 protein in 0.1ml at 2 weeks after the last dose of the hsp65 DNA immunisation and were read on days 1,3,5, 7, 14 & 21.

**M. tuberculosis $H_{37}Rv$**

| Gr | Immunised with | 2 wk. CFU Mean ± SD | 4 wk. CFU Mean ± SD | 6 wk. CFU Mean ± SD |
|---|---|---|---|---|
| I | pCMV(cont) | 1 5.80 ± 0.26 | 4 3.02 ± 1.79 | 7 2.98 ± 1.66 |
| V | HSP | 2 5.08 ± 0.22 | 5 2.95 ± 1.51 | 8 1.16 ± 1.45 |
| III | BCG | 3 2.34 ± 1.94 | 6 0.80 ± 1.60 | 9 0.96 ± 1.18 |

*P Value-*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1 Vs 2 | 0.009 | S | 4 Vs 5 | 0.954 | NS | 7 Vs 8 | 0.138 | NS |
| 2 Vs 3 | 0.047 | S | 5 Vs 6 | 0.086 | NS | 8 Vs 9 | 0.837 | NS |
| 1 Vs 3 | 0.023 | S | 4 Vs 6 | 0.102 | NS | 7 Vs 9 | 0.086 | NS |

**M.tuberculosis (Clinical isolate)**

| Gr | Immunised with | 2 wk. CFU Mean ± SD | 4 wk. CFU Mean ± SD | 6 wk. CFU Mean ± SD |
|---|---|---|---|---|
| II | pCMV(cont) | 11 4.37 ± 0.45 | 14 2.16 ± 1.79 | 17 0 |
| VI | HSP | 12 4.48 ± 0.46 | 15 0.82 ± 1.64 | 18 0 |
| IV | BCG | 13 3.47 ± 0.13 | 16 1.08 ± 2.16 | 19 0 |

*P Value-*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 11 Vs 12 | 0.775 | NS | 11 Vs 13 | 0.037 | S | 15 Vs 16 | 0.853 | NS |
| 12 Vs 13 | 0.028 | S | 14 Vs 15 | 0.411 | NS | 14 Vs 16 | 0.539 | NS |

Challenge: At 10 weeks after immunisation (10 weeks after the BCG and 6 weeks after the last dose of hsp65 DNA immunisation), Groups 1, 3 & 5 were challenged with *M. tuberculosis* $H_{37}Rv$, Groups 2, 4, & 6 were challenged with *M. tuberculosis* South Indian variant by intramuscular injection of 0.5ml of suspension of the respective organisms in distilled water containing 1mg of 2-week-old growth on L.J.

Sacrifice scoring and enumeration of CFU in spleen: At 2, 4 and 6 weeks after challenge, 4 guinea pigs from each group were sacrificed. Scores for the extent of infection were reported as per standardised scoring adapted by Mitchison et al. Spleen were removed, homogenised in 5ml of sterile distilled water using a teflon grinder and viable counts were set up by inoculating 10 µl of the resulting homogenate and five serial IO-fold dilutions on L.J.

Comparison of the protective efficacy of BCG and HSP65 DNA vaccines: The protective efficacies of the two vaccines were assessed and compared based on the reduction in the extent of infection and CFU in spleen in the animals vaccinated with BCG and the animals vaccinated with HSP65 DNA compared to the control animals.

The significant differences obtained at the end of 2 weeks compared to control with both the challenging strain, namely, *M. tuberculosis* $H_{37}Rv$ and South Indian clinical isolate were not observed at 4 and 6 weeks. Although CFU counts at 4 and 6 weeks in the groups injected with BCG and hsp65 DNA were lower than that of the control, a very high SD obtained at these time points negated this useful observation.

# APPENDICES

## Training Programmes

Post-graduate students, medical and para-medical personnel and technicians from other institutions in Chennai and outside underwent one- to four-week training in various departments of the Centre.

In addition, one- or two-day training programmes were arranged at the Centre for batches of medical students, post-graduates, nursing students and para-medical personnel.

## Staff Development Programmes

1. Dr. Mrs. H. Uma was awarded Ph.D. Degree from the Tamil Nadu Dr. MGR University for her thesis titled "Studies on the influence of human leucocyte antigens (HLA-DR & DQ) on immune responses in pulmonary tuberculosis" during January, 1998.

2. Dr. Jayasankar was awarded Ph.D. Degree from the Tamil Nadu Dr. MGR University for his thesis titled "Studies on the Biochemical and Histochemical changes relating to fibrosis following infection with *Mycobacterium tuberculosis"* during January, 1998.

3. Mr. Ravindra Rao underwent a two-month course at the National Tuberculosis Institute, Bangalore, on "Mass Miniature Radiography for District TB Programme" during January-March, 1998.

4. Mr. R. Subramani underwent training in "Internet Course" and "Web Site Development Course" at Videsh Sanchar Nigam Ltd., Chennai-600 002 during March, 1998.

5. Dr. (Mrs.) H. Shakila was awarded Ph.D. Degree from the Tamil Nadu Dr. MGR University for her thesis titled "Immunological studies in naturally occurring and experimental dermal granulomas induced by *Mycobacterium tuberculosis"* during March, 1998.

6. Dr. Soumya Swaminathan underwent training in both clinical and laboratory aspects of Paediatric HIV disease under FXB International Training Programme in Paediatriac HIV, University of Medicine and Dentistry, New Jersey, USA, during April, 1998.

7. Dr. R. Sattanathan was awarded Ph.D. Degree from the University of Madras for his thesis titled " A study of generalised additive models and their applications" during April, 1998.

8. Ms. Thresa Xavier, awarded a 9-month WHO Fellowship to study social work related tuberculosis and HIV at A.G.Holley State Tuberculosis Hospital, Lantana, Florida, USA during May, 1998.

9. Dr. Rajeswari Ramachandran. Dr. C. Kolappan and Dr.D. Vijaya Bhaskara Rao underwent 2-weeks WHO sponsored training programme on "Collection, management, analysis and presentation of data relating to tuberculosis" and training in "Epi Info" at National Tuberculosis Institute (NTI), Bangalore, during May, 1998.

10. Dr. D. Vijaya Bhaskara Rao and Mr. L. Sekar underwent 2-week training programme on "Controlled Clinical Trials" at Institute for Research in Medical Statistics (ICMR), Chennai during July, 1998.

11. Dr. K.S. Senthil Kumar was awarded Ph.D. Degree from the Tamil Nadu Dr.MGR University for his thesis titled "Purification of mycobacterial antigens and production of monoclonal antibodies to *Mycobacterium tuberculosis"* during July, 1998.

12. Dr. Rajeswari Ramachandran and Dr. Rani Balasubramanian underwent the programme on Training of RNTCP trainees, Puri, Orissa, organized by DANTB, Orissa, during August, 1998.

13. Mr.A.K. Hemanth Kumar underwent the training programme on developing HPLC separation conducted by 'WATERS" (India) Pvt. Ltd., Bangalore, during September, 1998.

14. Mr. P. Karthigayan attended the one week training programme on Visual Basic 5.0 conducted by Centre for Reliability (Govt. of India), Chennai during November, 1998.

# Papers Presented at Conferences

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| National Conference of the Indian Academy of Pediatrics, Cochin, 8-11 January, 1998 | Immunopathogenesis of tuberculosis | Dr. Soumya Swaminathan |
| 59rd Annual Conference of the Association of Physicians of India, Bangalore, 16-20 January, 1998 | Respiratory Diseases | Dr. V. K. Vijayan |
| Association of Physicians of India's Annual Conference, Bangalore, 16-20 January, 1998 | Rapid diagnosis of Tubelculosis | Dr. C. N. Paramasivan |
| International Conference on Population issues on the eve of the 21st Century, Varanasi, 9-12 February, 1998 | Age and sex structured model for measuring the demographic impact of HIV/AIDS in India | Dr. P. Venkatesan |
| First Annual Conference of the Association of Chest Physicians, Calcutta, 16 February, 1998 | Diagnosis and management of intestinal lung | Dr. V. K. Vijayan |
| Indian Association of Medical Microbiologist held at Madras Medical Mission, Chennai on 21 February, 1998 | Non-tuberculous Mycobacteria as an opportunistic pathogen | Dr. C. N. Paramasivan |
| Fifh International Congress on Cardio Pulmonary Diseases organised by the American College of Chest Physicians, Calcutta, 27 February-2 March, 1998 | Chronic respiratory infections | Dr. V. K. Vijayan (Chair person) |
| -do- | Tuberculosis in special situations | Dr.V.K. Vijayan |
| American Thoracic Society International Conference, Chicago, USA, 24-29 April, 1998 | Lymphocyte subpopulations and cytokine profiles in pediatric pulmonary tuberculosis | Dr. Soumya Swaminathan |
| National Conference on Recent Trends in Bio-technology & Microbial Research, Pudukottai, 20-21May, 1998 | PCR-SSCP in microbial research | Dr. N. Selvakumar |
| Hansens Diseases Centre, Louisiana State University, Baton Rouge, USA, 26 May-IO June. 1998 | Tuberculosis in India | Dr. C. N.Paramasivan |

(Contd...)

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| Center for Disease Control, Atlanta, 3 June 1998 | An overview on drug resistance surveillance in India | Dr. C. N. Paramasivan |
| National Conference on Health and Environment organised by the Centre for Science and Environment, New Delhi, 7-9 July, 1998 | Lung function assessment of Bhopal Gas Tragedy patients | Dr. V. K. Vijayan |
| APICON 1998 Conference conducted by the Karnataka branch of the API and the Cardiological Society of India, Bellary, 9 July, 1998 | Multi-drug resistant tuberculosis | Dr. M. S. Jawahar |
| Meeting of the Association of Physicians of India (API), Madurai, 26 July, 1998 | Basic immunology | Dr. V. Kumaraswami |
| Indian Medical Association, Chennai, 26 July 1998 | Therapy in chest medicine (Panel discussion) | Dr. V. K. Vijayan |
| Host pathogen defenses in *Mycobacterium tuberculosis* and HIV infection: Emerging Scenario, New Delhi, 10-11 August, 1998 | HIV and tuberculosis | Dr. Soumya Swaminathan |
| -do- | Multidrug resistance – tuberculosis management | Dr. Rajeswari Ramachandran |
| XIX Annual Conference of Women Doctors Association, Kancheepuram, 24-25 October, 1998 | Multidrug resistance overview including diagnosis | Dr. Rani Balasubramanian (Guest lecture) |
| International Symposium on Complement in Health and Diseases, New Delhi, 29- 31, October, 1998 | Complement component C3 in tuberculous granuloma | Dr. V. D. Ramanathan |
| -do- | Cellular immune response to *Mycobacterium tuberculosis* in children | Dr. Soumya Swaminathan |
| 10th International Congress of Immunology, New Delhi, 1-6 November, 1998 | The induction of a "Mitsuda" type response by purified protein derivative in tuberculosis | Dr. V. D. Ramanathan |
| -do- | Non-HLA gene polymorphism in pulmonary tuberculosis | Dr. P. Selvaraj |

(Contd...)

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| XXII National Congress on Indian Association of Medical Microbiologists at Kasturba Medical College, Manipal, 6 November, 1998 | Rapid methods for culture of Mycobacteria | Dr. C. N. Paramasivan |
| -do- | WHO/ IUATLD sponsored surveillance of drug resistance in tuberculosis | Dr. C. N. Paramasivan |
| -do- | Evaluation of the BACTEC radiometric method in the early diagnosis of tuberculosis | Mr. P. Venkataraman |
| -do- | Plasmid profile of *Mycobacterium fortuitum* complex | Daisy Vanitha |
| National Conference of Indian Chest and Respiratory Diseases Conference, Jallandher, 6-9 November, 1998 | Serumneopterin levels in pulmonary tuberculosis | Dr. Rajeswari Ramachandran |
| XVI Annual Conference of Indian Society for Medical Statistics, CMC, Vellore, 19-21 November. 1998 | A semi-parametric method for analyzing over- dispersed paired count data | Dr. P. Venkatesan |
| -do- | An assessment of the effectiveness of simple rules for the scrutiny of scientific reports | Dr. Vijaya Bhaskara Rao |
| 29th World Conference of the IUATLD Global Congress on Lung Health held at Bangkok, Thailand, 23-26, November 1998 | Surveillance of drug resistance in tuberculosis in Tamil Nadu, South India | Dr. C. N. Paramasivan |
| -do- | Patients' perceptions on public and private providers of TB services in India | Ms. Mohanarani Suhadev |
| 20th Biennial Conference of the Indian Associaciation of Leprologists, Gandhi Medical College, Bhopal, 28-30 November, 1998 | Objective parameters to assess the response to treatment in pauci-bacillary report | Dr. A. Thomas |
| Diamond Jubilee Symposia and 39th Annual Conference Association of Microbiologists of India, 5-7 December, 1998 | Recent advances in drug susceptibility testing of *M.* tuberculosis | Dr.N.Selvakumar |

(Contd.. .)

| Name of conference, venue and date | Title of paper | Name of staff member |
|---|---|---|
| International Workshop on Immuno-diagnostics sponsored by the Govt. of India, UNDP Programme organised bythe CDRI, Lucknow,7-12 December, 1998 | Tuberculosis:Epidemiology and Diagnosis | Dr. C. N.Paramasivan |
| 53rd National Conference on TB &Chest Diseases, Bhubaneswar, 27-30 December, 1998 | The influence of gender on action taking behaviour among sputum positive pulmonary tuberculosis patients | Dr. Rajeswari Ramachandran |
| -do- | Economic impact of tuberculosis on patients and family | Dr. Rani Balasubramanian |
| -do- | Impact of tuberculosis on private for-profit provider | Dr. R. Balambal |
| -do- | Action taken pattern among chest symptomatics from urban and rural areas | Mrs. Sudha Ganapathy |
| -do- | Sociological profile of pulmonary tuberculosis patients utilizing private health services | Mrs. Jaggarajamma |
| -do- | Treatment compliance in relation to sources of referral and initial action to the diagnosis of tuberculosis | Mrs. Beena Thomas |

# Participation in Symposia, Workshops and Training Courses

| Name of event, venue and date | Title of paper | Name of staff member |
|---|---|---|
| Informal consultation meeting on intensified action for TB control in SEAR, WHO/SEARO, New Delhi. 8-10 January, 1998 | The scenario of Tuberculosis Research | Dr. P.R. Narayanan |
| International Conference on Reproductive Health, AIDS Prevention and Development of Women organised by the Shree Venkateswara University, Tirupathy, 8-10 January, 1998 | | Mrs. Sudha Ganapathy |
| Refresher Course on "Recent trends in chemistry" conducted by the Dept. of Biochemistry, University of Madras, Chennai, 3-23, January, 1998 | | Dr. Prema Gurumurthy |
| Workshop on Intellectual Property Rights, IGCAR, Kalpakkam, 23 January, 1998 | | Dr. Alamelu Raja<br>Dr. Reetha Vijayan<br>Dr. Sujatha Narayanan<br>Dr. P. Venkatesan |
| Workshop on tuberculosis jointly organised by DST, ICMR, the European Commission and Indo-UK, Department for International Development at TRC, 3-5 February, 1998 | | Dr. P. R. Narayanan |
| Workshop on tuberculosis jointly organised by DST, ICMR, the European Commission and Indo-UK, Department for International Development at TRC, 3-5 February, 1998 | Environmental mycobacteria | Dr. C. N. Paramasivan |
| Workshop on tuberculosis jointly organised by DST, ICMR, the European Commission and Indo-UK, Department for International Development at TRC, 3-5 February, 1998 | Immunopathology of tuberculosis | Dr. V. D. Ramanathan |
| -do- | The modulation of immune response by BCG vaccination in South India | Dr. D. Sulochana |
| -do- | Identification of the promoter of acetmidase gene for expression of useful mycobacterial genes | Dr. Sujatha Narayanan |

(Contd.....)

| Name of event, venue and date | Title of paper | Name of staff member |
|---|---|---|
| Workshop on 'National Update on Asthma' organised by the Indian Asthma Care Society, Jaipur, 14-16 February, 1998 | Irritant induced asthma (Guest lecture) | Dr. V. K. Vijayan |
| One-day brain storming meeting at APAC of Public Voluntary Health Services, Chennai, 17 February, 1998 | | Dr. P. R. Narayanan |
| Conference on "Global Disease Elimination and Eradication as Public Health Strategies", Atlanta, 23-25 February, 1998 | | Dr. P. R. Narayanan (Chairman) |
| International Women's Day Celebrations organised by the MCCSS, Chennai, 12 March, 1998 | | Dr. Rani Balasubramanian<br>Dr. Geetharamani Shanmugam |
| Seminar on Community Initiatives for Tuberculosis Control, organised by the Tuberculosis Research Centre in collaboration with the Hindu, Chennai, 25 March, 1998 | | Dr. P.R. Narayanan |
| Refresher Course on Statistics for College Teachers conducted by the Academic Staff College, University of Madras, Chennai, March, 1998 | Analysis of Categorical data | Dr. P. Venkatesan |
| Workshop on Prevention of Spreading Rumours in STD/HIV/AIDS Control Programme at the Health and Family Welfare Training Centre, Chennai, 6 June, 1998 | | Mrs. Sudha Ganapathy |
| CME programme by Indian Academy of Paediatrics, Bangalore, 13 June, 1998 | Immunology of childhood tuberculosis (Guest lecture)<br><br>Recent advances in treatment of childhood tuberculosis | Dr. Soumya Swaminathan |
| ICMR sponsored Training Programme on Biostatistical Techniques in Controlled Clinical Trials organised by the Central Biostatistical Monitoring Unit for Traditional Medicine Research, Chennai, 13-24 July, 1998 | | Dr. T. Santha<br>Dr. V. Kumaraswami<br>Dr. M. S. Jawahar<br>Dr. P. Venkatesan (Faculty Members) |

(Contd....)

*(Continued)*

| Name of event, venue and date | Title of paper | Name of staff member |
|---|---|---|
| "Workshop on Control of Tuberculosis – New Strategies" organised by Advocacy for Control of Tuberculosis, TRC and The Hindu, 25 July, 1998 | | Dr. P. R. Narayanan<br>Dr. T. Santha Devi<br>Dr. V. Kumaraswami<br>Dr. M.S. Jawahar |
| Seminar on Frontiers in Luminescence at IGCAR, Kalpakkam, Chennai, on 24 August, 1998 | Bioluminescence assay using luciferase reporter phage | Dr. Vanaja Kumar |
| World Asthma Meeting, Barcelona, Spain, 9-13 September, 1998 | | Dr. Soumya Swaminathan |
| Second meeting of the Apex Committee of VAP, New Delhi, 18 September, 1998 | Overall scenario on emergence of tuberculosis, epidemiology and drug resistance | Dr. P. R. Narayanan |
| Symposium on "New and Emerging Diseaes Syndrome" along with Joint Working Group (JWG) meeting of the Indo-US VAP, Washington, 5-7 October, 1998 | Overall scenario on emergence of tuberculosis, epidmiology and drug resistance | Dr. P. R. Narayanan |
| Workshop on "Research Methodology and Bio-statistics" conducted by the Tamil Nadu Dr. MGR Medical University Chennai, 23-26 November, 1998 | | Mrs. Geetha Ramachandran<br>Mr. A. K. Hemanth Kumar<br>Miss. B. Priya |
| Avinashilingam Institute of Home Science and Higher Education for Women – Deemed University, 21 November – 21 December, 1998 | Course on Biotechnology | Dr.Sujatha Narayanan |
| 4th National Bronchology Conference, Chennai, 15-17 December 1998 | | Dr. Soumya Swaminathan |

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1. Das, S., Narayanan, P.R., Kolappan, C., and Colston, M.J.
The cytokine response to Bacille Calmette Guerin vaccination in South India.
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3. Jawahar, M.S.
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4. Paramasivan, C.N.
Laboratory diagnosis of childhood tuberculosis (Editorial). *IAP Journal of Practical Paediatrics,* 1998,4,77-79.

5. Paramasivan, C.N.
Study on environmental Mycobacteria obtained from South Indian BCG trial area. Published in the Proceedings of the Workshop on "Tuberculosis Research : In to the 21st Century ", 1998,75-81.

6. Paramasivan, C.N., Kubendiran , G., and Herbert, D.
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17. Selvakumar, N., Ding, B.C., Ruth McNerney, Stuart M. Wilson, and Narayanan, P.R.
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21. Shenoy, R.K., Verghese, J., Kutlikkal, V.V., and Kumaraswami, V.
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Treatment of microfilaraemia in asymptomatic brugian filariasis: the efficacy and safety of the combination of single doses of ivermectin and diethylcarbamazine. Annals of Tropical Medicine & Parasitology, 1998, 92, 579-585.

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28. Venkataraman, P., Herbert, D., and Paramasivan, C.N.
Evaluation of the BACTEC radiometric method in the early diagnosis of tuberculosis. *Indian Journal of Medical Research,* 1998,108,120-127,

29. Vishwanath, Sujatha Narayanan, and Narayanan, P.R.
The fate of *Mycobacterium tuberculosis* in activated human macrophages. Current Science, 1998; 75,936-939.

# Guest Lectures & Journal Club Meetings

## Background

*Journal Club Meetings were held each week, at which published scientific articles covering different areas of research were reviewed by staff members of various departments in turn. A synopsis of the paper(s) to be presented and the reference details were circulated in advance, to facilitate better participation by the audience in the discussion that followed the presentation. In all, 47 such meetings were conducted during the one year in (1998) including 8 guest lectures and 4 lectures by the staff of IRMS.*

## *Speakers '98*

Prof. Sunnyluke, Associate Director of Research, Department of Pathology, Maimonides Medical Centre, Brooklyn, New York, "Current Status of Fluorescence in situ hybridization(FISH) in diagnostic pathology"

Mr. Jnani. Journalist, "Health and Communication"

Dr. James Nelson, Vice-president, Molecular Devices, Spinco Biotech Ltd., USA, "Jmax, Flipa and Cyto-sensor"

# Distinguished Visitors

Dr. (Mrs.) Ira Ray, Additional D.G.H.S. & Director of NIB, Ministry of Health, Govt. of India, New Delhi.

Dr. S. P. Rajagopalan, Member Syndicate and Principal of D.G. Vaishnava College, Chennai

Dr. Mrs. Saraljith Sehgal, Additional Director General, Directorate of Health Services, New Delhi.

Dr. S. K. Satpathy, Senior Adviser, DANTB, Bhuvaneswar – 751 015.

Mr. R.K. Sharma, Commissioner, Indian Medicine, Chennai.

| | | SPEAKER |
|---|---|---|
| 27-01-98 | *Gene to vaccine* | **Prof.P.Kaliraj,**<br>Director, Centre for Bio-technology,<br>Anna University,Chennai. |
| 13-04-98 | ***Risk factors in human cancer*** | **Dr.B. Nagarajan,**<br>Head, Department of Microbiology & Tumour Bio-chemistry,Cancer Institute, Adyar,Chennai. |
| 13-07-98 | *Journey in search of science and research* | **Dr.H.Srinivasan,**<br>Hon. Editor, Indian Journal of Leprosy, Chennai. |
| 30-07-98 | *Eye donation* | **Mr.A.P.Irungovel,**<br>Social Worker, Sanakara Nathrayalaya,<br>Nungambakkam, Chennai. |
| 11-08-98 | ***Blood components:Their usefulness and effctiveness*** | **Dr. Anitha Suriyanarayanan,**<br>Chief Medical Officer,<br>Jeevan Blood Bank,<br>Nungarnbakkam, Chennai. |
| 11-08-98 | ***Blood donoation: Some truths*** | **Dr.P.Srinivasan,**<br>Director, Lister Laboratory, Nungambakkam, Chennai. |

# Workshops organised by TRC jointly with national & international agencies

## TRC - "THE HINDU"

### Advocacy for the control of Tuberculosis (ACT)

Tuberculosis for which effective interventions exist, remains a major public health problem. A century after the discovery of the TB bacilli and fifty years after the discovery of powerful drugs we find ourselves losing our battle against mankind's leading killer. The introduction of the concept of Directly Observed Treatment Short course (DOTS) has shifted the responsibility of completing treatment from the patient to the provider which in most cases, the private practitioner. It is clear that the WAR against TB is primarily an advocacy challenge rather than a medical challenge. Recognising the reach and commitment of private practitioners in the community and the vital role that they play in the control of tuberculosis in the community the Tuberculosis Research Centre in association with "THE HINDU" set up a forum called ACT (Advocacy for the Control of Tuberculosis) to share experiences and to develop collective Wisdom with regard to TB awareness and TB advocacy. The inaugural meeting on 25th March, 1998 was chaired by the renowned agricultural scientist Dr. M. S. Swaminathan.

Under the aegis of ACT, over 50 physicians met on different occasions to discuss problems relating to TB control.

These interactive meetings demonstrated their recognition of tuberculosis as a major health problem and their desire to be part of a committed group to fight this dreaded disease. In these sessions they agreed that the diagnosis of tuberculosis should necessarily be made on more measurable parameters such as smear examination for the tubercle bacilli. They also recognised that the documentation of their successes would help others learn from this experience of practising the "right way to treat tuberculosis".


Workshops were held to familiarise the participating physicians with the concepts of diagnosis, initiation of treatment and followup under the DOTS programme.

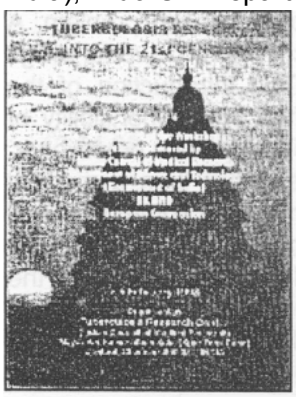
Simultaneously. laboratory technicians attached to 21 private clinics were trained to carry out sputum smear examinations at the Tuberculosis Research Centre. Their performance was then evaluated by periodic quality control checks and found to be satisfactory.

Currently nearly 50 private practitioners assoicated with ACT are enrolled in a programme to practice DOTS in the city of Chennai. This marks the begining of a new phase in the control of tuberculosis which harnesses the potential of the private sector.

# TRC - DST - Indo-EU - DFID *(U.K.)*

## Tuberculosis Research: Into the 21st Century

A three day Workshop on Tuberculosis entitled "TUBERCULOSIS RESEARCH: INTO THE 21st CENTURY" jointly sponsored by the Indian Council of Medical Research (Ministry of Health and Family Welfare, Govt. of India), Department of Science and Technology (Govt of India), Indo-UK Department for International Development (DFID) and the European Union



(EU) was held at Chennai from 3rd to 5th February 1998. The Tuberculosis Research Centre organised the workshop on behalf of all the sponsoring agencies.

This workshop highlighted the current trends in research in Tuberculosis and identified newer areas for exploration. It brought together leading British and other EU scientists to interact with their Indian counterparts to exchange their views and identify areas for further research. A major highlight of the workshop was the presentation of the data and impact assessment of the DFID funded project on tuberculosis.

Taking cognizance of the major scientific breakthroughs in tuberculosis research over the past years, this workshop was organised around various topics of importance in the field. Special attention has been given to modern molecular genetics, the tuberculosis, genome project, new drug and vaccine development and cellular immunology.

It provided an opportunity for the scientists to present their concepts and data during their lectures which were followed by critical discussions. There was a unanimous opinion that DOTS is the best help that is available today, but it alone is insufficient to reduce tuberculosis incidence appreciably on a Global scale. The highlights of the workshop were the recommendations made by the participants for future research and an agreement to share knowledge and experience with a common goal of controlling the most dreaded disease of mankind.

The proceedings of the Workshop was released by Prof. N. K. Ganguly, Director General, ICMR at a function held at TRC on 16 August 1998.

Recommendations of the Workshop

1. *Establishing both multilateral and bilateral funding programmes for collaboration between Indian and European groups in the field of tuberculosis.*
2. *Studies should focus on MDRTB and the association between HIV infection and tuberculosis*
3. *Functional genomics of mycobacteria need to be studied extensively.*

## Women In Science - A Poster Symposium

A poster symposium entitled "Women in Science" was held on 7th April, 1998 as part of the 50th year celebrations of India's Independence.

Poster symposium was held with a view to highlight the achievements of women candidates registered for their Ph.D. in various diciplines ranging from Chemistry to Physical Sciences to Life Sciences.

The inagural address was delivered by Dr. Lalitha Kameswaran, Retd. Vice Chancellor of Sri Ramachandra Medical College and Research Institute, Dr. Kunthala Jayaraman, Dean of Anna University gave the Presidential address and released the souvenir entitled "Women in Science". Dr. Nazareth, retired Medical Officer from TRC, graced the occasion.


The crossword quiz contest based on the abstracts presented for the poster session was a big hit among the participants. It ensured that every poster got its due attention! The quiz competition based on the theme "Women achievers in various fields" kept the participants and the audience enthralled. Dr. Nalini Krishnan, Director of the "The Hindu" conducted the valedictory function and distributed the prizes.

## ACKNOWLDEGEMENT

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