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CELL MEDIATED IMMUNE RESPONSE IN SOUTH INDIAN PULMONARY TUBERCULOSIS PATIENTS

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Abstract : Ninety-two untreated pulmonary tuberculosis (TB) patients and sixty two non-tuberculous (non-TB) controls drawn from the same socio-economic strata were studied for their delayed hypersensitivity (DH), cell mediated immunity (CMI) and bacillary-load. There was no correlation between bacillary load and response in parameters of CMI, namely, lymphocyte transformation test (LTT) and leucocyte migration inhibition test (LMI). Migration Index (MI) in LMI did not correlate with DH in TB patients. There was no significant difference in the mean values of MI between TB and non-TB control patients. Response of lymphocytes to mitogens and percentage of T-rosetting cells also did not differ between TB and non-TB control patients. On the contrary, differences were found in the two groups of patients with regard to lymphocyte response to PPD antigen and mean B-cell percentage. While, among non-TB patients, high Mantoux reactors had significantly higher LTT response to PPD compared to low Mantoux reactors; no such difference was observed among TB patients. The mean B-Cell percentage was significantly higher in TB patients than in non-TB controls. The relevance of these differences in relation to tuberculosis is discussed.

Introduction

A number of studies have been carried out to assess the immunological responses of pulmonary tuberculosis patients. However, there is no consensus on the exact nature of the defect in the immune system of these patients. Lenzini, *et al.* (1977) suggested a spectrum of immunological responses in human tuberculosis. At one end of this spectrum is miliary and highly advanced pulmonary tuberculosis marked with depressed cell mediated immune response (CMI). The other end of the spectrum is represented by primary complex which is associated with well developed CMI responses. Bhatnagar *et al.* (1977) studied immune response in North Indian patients. Their results also suggest the presence of an immunological spectrum in tuberculosis patients. The cell mediated immune responses have not been studied in South Indian pulmonary tuberculosis patients. In view of the fact that South Indian patients are more often infected with a low virulence strain of *M. tuberculosis* (Bhatia *et al.*, 1961), it was thought that their cell mediated immune reactions may be different from those reported in other studies. Cell mediated immune responses of South Indian pulmonary tuberculosis patients are compared with controls belonging to same socio-economic strata.

Materials and Methods

Patient population : Ninety-two untreated pulmonary tuberculosis (TB) patients attending this Centre were admitted to the study. All the patients were bacteriologically confirmed

by sputum microscopy for acid fast bacilli (AFB) and culture for *M. tuberculosis*. Sixty-two non-tuberculous (non-TB) controls were admitted from the out-patient clinic of Institute of Tuberculosis and Chest Diseases (I.T.C.D.), Madras. They were examined for tuberculous disease by a chest x-ray and sputum smear. Only patients with x-rays not suggestive of TB and negative sputum smear were admitted.

Mantoux Reaction : The TB patients and controls were subjected to Mantoux test with 1 T.U. of PPD RT23 (BCG Laboratory, Guindy, Madras, India). The transverse diameter of induration was read at 48-72 hours. Generally, an induration of 10 mm or more is considered as an evidence of present or past infection and is taken as positive response. However, due to the wide prevalence of atypical mycobacteria in this area (Paramasivan *et al.*, 1985) individuals with reaction of 16 mm and more were considered Mantoux high reactors. All tuberculous patients with Mantoux reaction of 15 mm or less and about equal number of patients with Mantoux reaction of 16 mm and above were admitted to the study.

Immunological Investigation : Immunological tests were carried out on 20 ml heparinized blood collected prior to start of chemotherapy. All TB patients were examined by chest x-ray and colony forming units of viable bacilli from a pretreatment specimen of sputum collected overnight.

Lymphocyte transformation test : Peri-

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peripheral blood lymphocytes (PBL) were purified on Ficoll-hypaque (1.071 sp. gravity) density gradient as described by Boyum (1968). They were washed thrice in Hank's balanced salt solution and finally cultured in 96 well micro-culture plate (Dynatech Corp.) in 0.2 ml tissue culture medium RPMI-1640 (containing 100 IU/ml Penicillin, 100 ug/ml Streptomycin, and 10% autologous plasma). Cultures were stimulated with either 10 ug/ml of Phytohaemagglutinin-A (PHA, Burroughs Wellcome Labs, U.S.A.), or 10 ug/ml of Poke weed mitogen (PWM, Sigma Chemical Co., U.S.A.) or 10 or 25 ug/ml of PPD-298 (Purified Protein Derivative of *M. tuberculosis*, Ministry of Agriculture Food & Fisheries, Weybridge, U.K.). Stimulated cultures were maintained for 4 and 6 days for mitogen and antigen respectively. One μCi ^3H -Thymidine was added to each culture well one day before harvesting. ^3H -Thymidine was measured in liquid scintillation counter (LSS-20, ECIL, Hyderabad, India). The response was represented as Stimulation Index (S.I).

$$\text{S.I.} = \frac{\text{Mean of Log counts in Stimulated culture}}{\text{Mean of Log counts in Control culture}}$$

T and B cell Rosettes : T cell rosettes were performed according to the method described by Jondal *et al* (1972) and Paranjape *et al* (1986). Procedure for B-rosetting was modified from Bianco *et al* (1970). In brief, 5% suspension of sheep red blood cell (SRBC) was incubated with 1/200 dilution of rabbit anti-SRBC serum at 37°C for 15 minutes. Sensitized SRBC, were then washed thrice and resuspended to 5% and incubated with equal volume of 1/10 dilution of guinea pig serum as a source of complement, The cells were

washed after one hour incubation and resuspended to 0.5% concentration. 0.2 ml of this suspension was incubated with 0.2 ml peripheral blood lymphocytes (PBL) for 15 minutes. Rosette forming cells were counted and the percentage of rosette forming cells was determined as a mean of triplicates.

Leucocyte Migration Inhibition : The direct leucocyte migration test was performed according to the procedure described in Bendixen and Soborg (1969). PPD and *M. tuberculosis* H₃₇Ra suspension (40 ug/ml) were used as the antigens. Migration chambers (with capillaries with cell pellets) were incubated at 37°C for 18-24 hours and area of migration was plotted on Whatman No. 1 filter paper using projection microscope. The filter papers were cut as per area of migration and weighed. The migration index (MI) was calculated as :

$$\text{MI} = \frac{\text{Mean weight of migration area in presence of antigen}}{\text{Mean weight of migration area in absence of antigen}}$$

A migration index of 0.80 or less was considered to denote positive reaction.

Results

Mean ages of TB and control patients are shown in Table-1 by sex and size of Mantoux induration. The age among females ranged from 15-50 years in TB patients and from 13-65 years in non-TB patients. Among the males, the age ranged from 14-75 years in TB patients and from 20-55 years in non-TB patients.

Table I
Age, Sex and numbers of patients and controls

Group	Mantoux induration (mm)	Male		Female		All	
		No.	Mean age	No.	Mean age	No.	Mean age
TB Patients :							
Low Reactors	0 – 15	39	37	4	25	43	36
High Reactors	≥ 16	34	33	15	30	49	32
	All	73	35	19	29	92	34
Non-TB Controls :							
Low Reactors	0 – 15	19	25	11	33	30	28
High Reactors	2 – 16	21	30	11	28	32	29
	All	40	28	22	30	62	29

T and B cells : The mean values of T and B cell percentages are presented in Table 2. Mean T-cell percentage in non-TB patients (40%) was not significantly different from that in TB patients (34%). On the contrary, the mean B-cell percentage of 29% in TB patients was significantly higher than the mean percentage of 15% in non-TB patients ($P < 0.05$). Mean T and B cell percentages of Mantoux low reactors and high reactors in each group were practically same.

Leucocyte migration inhibition test : TB patients and controls had mean M.I. to H_{37} Ra of 0.87 and 0.88 with 42% and 51% reactors respectively (Table 2). Similarly, against PPD both TB patients and Non-TB controls had mean M.I. of 1.00 with 29% and 22% reactors respectively. Table 3 shows comparisons on the basis of Mantoux reaction as well as tuberculous disease. In TB patients, high and low Mantoux reactors had mean values of 0.96 and 1.04 respectively, for PPD, and 0.84 and 0.90 respectively, for H_{37} Ra antigen. Mantoux high reactor and low reactor Non-TB controls had mean M.I. (H_{37} Ra) of 0.81 and 0.96 respectively; however, the difference was not statistically significant.

Lymphocyte transformation test : The mean S.I. for PHA in low and high Mantoux reactors in TB patients was 1.48 and 1.53, respectively. Low and high mantoux reactor controls had mean S.I. of 1.50 and 1.57, respectively (Table 3). The response to PWM did not differ much between either TB patients and non-TB controls or high Mantoux reactors and low Mantoux reactors.

The mean S.I.s to 10 ug of PPD in high and low Mx reactor TB patients were 1.06 and 1.07 (Table 3). The response was similar with 25 ug/ml of PPD. High Mantoux reactor non-TB controls showed significantly higher mean S.I. to both 10 and 25 ug/ml of the stimulating dose of PPD (1.26 ± 0.25 and 1.19 ± 0.25) Low Mantoux reactor Non-TB controls had mean stimulation index comparable to that of TB patients (1.08 ± 0.13 and 1.04 ± 0.16). The difference between high and low Mantoux reactor non-TB controls was statistically significant ($P \leq 0.05$).

Mantoux reaction and parameters of immune response : It can be seen from Fig. 1 that in tuberculosis patients there was no correlation between Mantoux reaction and the parameters of CMI. In non-TB controls, though there

Table 2

Immunological responses in tuberculosis patients and non-tuberculous controls

Tests	Pulmonary tuberculosis		Non-TB controls	
	n	Mean	n	Mean
T-cells (%)	72	34	49	40
B-cells (%)	73	29	46	15
LMI				
Mean Migration Index to :				
PPD	69	1.00	49	1.00
H_{37} Ra	69	0.87	47	0.88
Percentage of reactors to :				
PPD	69	29%	49	22%
H_{37} Ra	69	42%	47	51%
LTT				
Mean Stimulation Index to :				
PHA	73	1.51	43	1.53
PWM	66	1.45	41	1.44
PPD 10 ug/ml.	69	1.07	36	1.18
PPD 25 ug/ml.	69	1.06	36	1.12

Table 3

Comparison of cell mediated immune response between high and low Mantoux reactors in tuberculous patients and non-tuberculous controls

Test of CMI	Low Mantoux Reactors (0-15 mm)				High Mantoux Reactors (16mm and above)				
	TB		Non-TB		TB		Non-TB		
	n	Mean	n	Mean	n	Mean	n	Mean	
Lymphocyte Trans-formation (S.I.) :									
PHA	34	1.48	23	1.50	39	1.53	20	1.57	
PWM	29	1.46	22	1.39	37	1.45	19	1.50	
PPD 10ug	33	1.06	17	1.08	36	1.07	19	1.26	
PPD 25ug	33	1.07	17	1.04	36	1.06	19	1.19	
LMI (MI) :									
PPD	32	0.96	24	0.99	37	1.04	25	1.01	
H ₃₇ Ra	32	0.84	22	0.96	37	0.90	25	0.81	
LMI (% Reactors) :									
PPD	32	31%	24	29%	37	27%	25	16%	
H ₃₇ Ra	32	50%	22	41%	37	35%	25	60%	

was no direct correlation between Mantoux induration and any of the parameters, there was group wise association between high Mantoux reactivity and high S.I. to PPD (Table 3). High mantoux reactor controls had higher proportion of high S.I. to PPD.

Bacillary load and immune response : Fig 2 shows the scatter diagram between log₁₀ viable counts of *M. tuberculosis* in overnight sputum collection and T-Cell percentage, M.I. to H₃₇Ra and S.I. to PPD. There was no correlation between log viable counts, i.e., bacillary load, and parameters of CMI.

Discussion

Several investigators have studied the relationship between Mantoux reactivity and Migration Index in Pulmonary TB Patients, and reported different findings. Bhatnagar *et al.* (1977), Lenzini *et al.* (1977) and Agnihotri, *et al.* (1978), reported good correlation, while Riger *et al.* (1979) found no correlation between the two tests. Rosenberg and David (1970) did not find any correlation in Pulmonary TB patients; but, found some association between these two parameters in non-TB patients. In this study, using PPD and *M. tuberculosis* H₃₇Ra live suspension as antigens, there was no correlation between tuberculin reactivity and MI in South Indian Pulmonary TB patients (Fig. 1). Only suggestive association (statistically

not significant) between the two parameters was found in non-TB controls.

In this study, pulmonary tuberculosis patients had significantly higher mean B-cell percentage than non-TB controls. But, there was no difference between the two groups in T-cell percentages. Increase in B-cell percentage was reported by Bhatnagar *et al.* (1977) in miliary tuberculosis but not in pulmonary tuberculosis, while Skvor and Trnka (1979) and Bhatnagar (1977) reported decrease in T-cell percentage in pulmonary tuberculosis patients.

The lymphocyte proliferative response to specific stimulus (PPD) was low in tuberculosis patients. In non-TB controls there was difference in S.I. between high and low mantoux reactors. The mean S.I. in pulmonary tuberculosis patients was 1.07. High reactors non-TB controls (Mx ≥ 16mm) had significantly higher mean S.I. to PPD than in low reactor controls (P<0.05). We believe that this positive association between Mantoux reactivity and lymphocyte proliferative response to PPD antigen in non-TB controls provides evidence of an immunological phenomenon (mechanism), particularly in view of the fact that such association was not found in tuberculosis patients.

The lack of response in high Mantoux

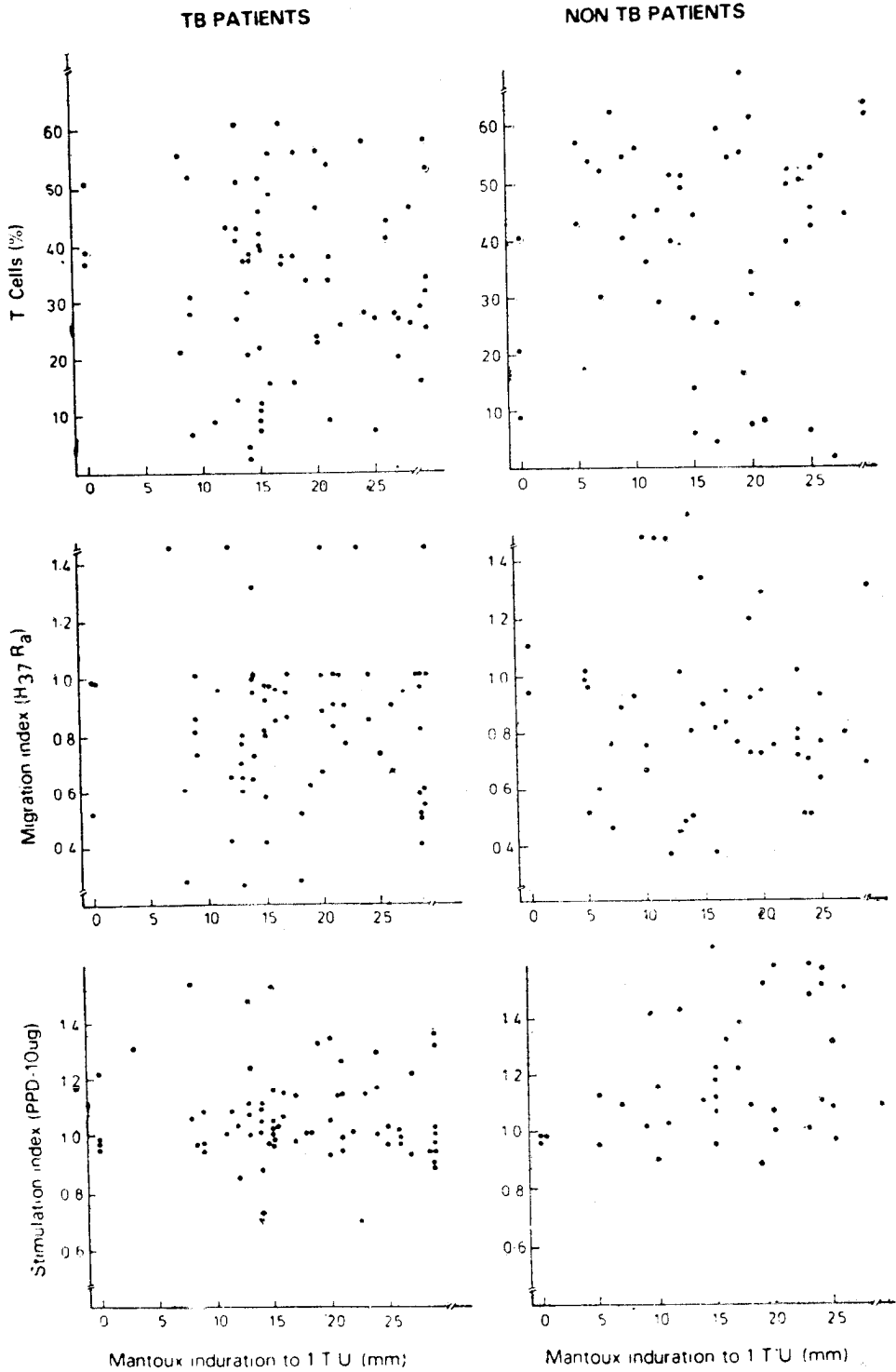


Fig. 1

The scatter diagrams in this figure show correlation between Mantoux induration (in mm) and parameters of CMI (T cell percentage, CMI to H₃₇Ra antigen and S.I. to PPD) in tuberculosis patients and non-tuberculous controls.

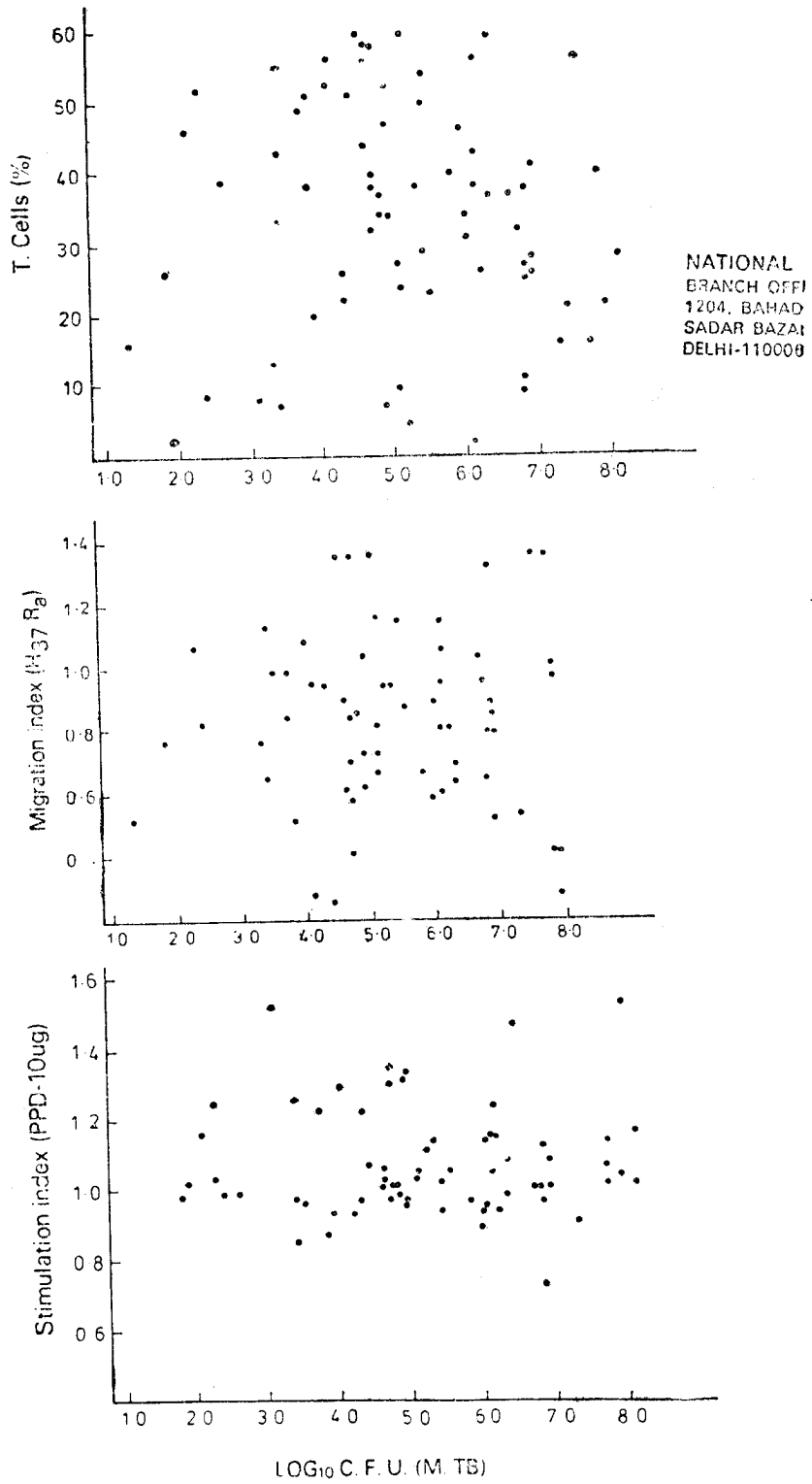


Fig. 2

Figure shows scatter diagram between Log₁₀ C.F.U. of *M. tuberculosis* in overnight collection of sputum and parameters of CMI (T-cell percentage, M.I. to H₃₇Ra antigen and S.I. to PPD).

reactor TB patients may be due to sequestration of specific committed lymphocytes to the site of infection, where bacterial, i.e., antigenic load is usually enormous. It is also possible that humoral and cellular suppressor mechanisms are involved in tuberculosis. Ellner (1978a, b) has reported the presence of antigen specific and adherent suppressor cells. Such suppressor phenomenon has been well documented in lepromatous leprosy patients Mehra, V. et al. 1980; Nath, I and Singh, R. 1980).

The differences between the results of studies referred above and this study may arise because of differences in clinical and bacteriological classifications of tuberculosis patients. For example, most of our patients may fall in the category 'unreactive' or 'unreactive intermediate' in Lenzini's spectrum of tuberculosis patients. In their study, 95% in these categories were Mantoux negative and they did not respond to treatment, whereas, about 80% of patients in this study were Mantoux reactive and practically all of them responded favourably to the treatment. Association between LMI response and the quantity of mycobacterial antigen in the body was reported by Skvor and Trnka (1979). However, bacillary load as indicated by viable counts of *M. tuberculosis* in sputum was not associated with CMC responses in this study.

The type of controls selected for the study also have bearing on the results obtained. In most other studies, normal healthy subjects who did not belong to the same socio-economic strata as patients were chosen as controls (Bhatnagar et al. 1979; Lenzini, 1977). In this study controls were drawn from same socio-economic strata. Considering the differences in the design, disease status of patients, type of controls and other factors, each study has to be carefully evaluated. This study and most other studies, however, do report defective immune response in TB patients. We found that association between Mantoux reaction and other parameters was evident in non-TB controls, but was absent in TB patients. TB patients with high Mantoux reactivity show low response in lympho-proliferative assays. Is it possible that T-cell population responsible for lympho-proliferative response is reduced in pulmonary tuberculosis? Or, is it the result of sequestration of specifically committed T-cells at the site of infection. It will also be interesting to know whether the Mantoux reactivity, after successful chemotherapy, would show association with parameters of immunity as seen in the control patients.

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REFERENCES

- Agnihotri, M.S., Chaturvedi, U.C. and Pande, S K. Immunological classification of pulmonary tuberculosis. *Ind J. Tub., 1977, 25 : 65-76.*
- Bendixen, G. and Soborg, M.A. leucocyte migration technique for in vitro detection of cellular (delayed) type hypersensitivity in man. *Dan. Med. Bull., 1969, 16, 1.*
- Bhatanagar, R., Malaviya, A.N., Narayanan, S., Premavathy Rajagopalan, Kumar, R., and Bhara-dwaj, O.P. Spectrum of immune response abnormalities in different clinical forms of tuberculosis, *Amer. Rev. Res. Dis, 1977, 115 : 207-212.*
- Bhatia, A.L., Csillag, A., Mitchison, D.A., Selkon, J. BZ., Somasundaram, P.R. and Subbaiah, T.V. The virulence in the guinea-pig of tubercle bacilli isolated before treatment from South Indian patients with pulmonary tuberculosis. 2. Comparison with virulence of tubercle bacilli from British patients. *Bull. WHO, 1961, 25 : 313-.*
- Bianco, C., Patric, R. and Nussenweig, V.A. Population of lymphocytes bearing a membrane receptor for antigen-antibody complement complexes. I. separation & characterization. *J. Exp. Med. 1973., 132 : 702.*
- Bovum, A. Separation of leucocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest., 1968, 21 (Suppl-97) : 77.*
- Ellner, J.J. Antigen specific suppressor cells in human tuberculosis (abstract) *Clin. Res., 1978, 26 : 523A.*
- Ellner, J.J. Suppressor adherent cells in human tuberculosis. *J. Immunol., 1978, 121 : 2573.*
- Jondal, M., Holm, G., and Wigzell, G.H. Surface markers on human T & B lymphocytes : I. Large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *J. Exp. Med., 1972, 136 : 207.*
- Lenzini, L., Rottoli, P. and Rotteli, L. The spectrum of human tuberculosis, *Clin. Exp. Immunol., 1977. 27 : 230.*
- Mehra, V., Mason, L H., Rothman, W., Reinherz, E., Schossmen, SF, and Bloom, B R. Lepromin induced suppressor cells in patients with leprosy. *J. Immunol., 1980, 123 : 1813.*
- Nath, I. and Singh, R. The suppressive effect of *M. leprae* on the in vitro proliferative responses of lymphocytes from patients with leprosy. *Clin. Exp. Immunol. 1980, 41 : 406.*
- Paramasivan, C.N., Govindan, D., Prabhakar, R., Somasundaram, P.R., Subbammal, S. and Tripathy, S.P. Species level identification of non-tuberculous mycobacteria (NTM) from South Indian trial area during 1981, *Tubercle, 1985, 66 : 9.*
- Paranjape, R.S., Acharyulu, G.S., Krishnamurthy, P.V., Ramachandran, P., Ravooof, A., Narayanan, P.R., Tripathy, S.P. and Prabhakar, R. Cell mediated immunity in tuberculosis meningitis, *Indian pediatrics, 1986, 23 : 127.*

- Rieger, M., Tmka, L. and Skvor, J. Immunoprofile studies in patients with pulmonary tuberculosis. IV. Tuberculin skin reaction and test of inhibition of blood leucocyte migration. *Scand. J. Resp. Dis*, 1979, 60 : 355.
- Rosenberg, S.A. and David, J.R. Inhibition of leucocyte migration. An evaluation of this assay of delayed hypersensitivity in man to a soluble antigen ? *J. Immunol.* 1970, 105 : 1447.
- Skvor, J. and Trnka, L. Immunoprofile studies in patients with pulmonary tuberculosis I- correlation of pretherapy cellular tests with characteristics of the disease. *Scand. J. Resp. Dis.*, 1979, 60 : 161.

The XVIth Conference of the Eastern Region of the IUAT-LD will be held in Beijing (Peoples Republic of China) from 20th to 23th September, 1989. Those interested in attending the Conference and/or presenting papers may contact the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001. Last date for sending four copies of the abstracts is 31st March, 1989.