IMMUNE RESPONSE PROFILE IN PATIENTS WITH ACTIVE TUBERCULOSIS IN A BCG VACCINATED AREA

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Abstract. Tuberculosis patients with pulmonary (N = 95) or lymph node disease (N = 23) were assessed for Th1 responses (PPD skin test and lymphocyte blastogenic and interferon γ) and Th2 responses (polyclonal and antigen specific IgE). Skin test responses to PPD and lymphocyte proliferative responses to crude mycobacterial antigens (PPD, culture filtrate and sonicate) and recall antigens (tetanus toxoid and streptolysin O) were significantly suppressed ($p < 0.001$) in patients with pulmonary disease compared to endemic controls. However, mitogen (phytohemagglutinin) - stimulated responses were comparable in patients and controls. Polyclonal and antigen specific (*M. tuberculosis* culture filtrate) IgE responses which are considered to be surrogate markers for Th2 responses were significantly higher in patients with pulmonary disease compared to healthy endemic controls (Mann Whitney analysis $p < 0.01$). Patients with lymph node disease showed strong Th1 responses but did not show significant responses for either polyclonal or antigen specific IgE. Thus overall suppression of T cell memory response was observed only in patients with pulmonary disease but not in patients with lymph node disease suggesting that sequestration of antigen in different compartments leads to differential activation of Th1 and Th2 responses. PPD skin test responses were highly positive in endemic controls (47% positive) and household contacts (86% positive). Furthermore, PPD positivity decreased with disease severity. Therefore PPD positivity in a BCG vaccinated TB endemic area cannot be used as a diagnostic marker for active tuberculosis particularly in advanced disease.

INTRODUCTION

Tuberculosis (TB) has always been a major problem for the developing world as one of the leading causes of death due to a preventable disease. World Health Organization estimates that *Mycobacterium tuberculosis (M.tb.)* infects 1/3 of the world population and accounts for 6.7% of all adult deaths, the majority of which occur in the developing world. In 1990 deaths estimated by WHO from various infectious and parasitic disease were 1.9 million due to tuberculosis compared to 0.2-0.4 million due to diarrhea, malaria, and other tropical diseases in decreasing order (Source: World Bank, World Development Report, 1993). It is expected that if this situation persists, thirty million people will die from TB in the next decade. This situation exists despite the availability of effective antituberculous drugs as well as a BCG vaccine. The emerging drug resistance may be a contributing factor to the increased incidence of tuberculosis, particularly in TB endemic areas. However, the reasons for the variable efficacy of BCG vaccination in different populations is still not clear. Eight major prospective BCG vaccine trials have been carried out to assess the protective efficacy of BCG vaccination in various populations. While BCG vaccination decreased the incidence of disseminated TB quite effectively, in the case of pulmonary tuberculosis the protective efficacy varied from 0-80% in different popula-tions and this was not related to differences in vaccine lots, racial differences or environmental differences (Fine, 1988). The main message that comes from these studies is that we still lack an understanding as to what constitutes protective immunity, despite tremendous strides in the field of immunology. The rational design of a more effective vaccine against tuberculosis requires a better understanding of the pathogenesis of infection particularly during the early stages of infection and establishment of disease. The current study addresses this issue.

Pakistan is a highly endemic area for tuberculosis with an estimated incidence of 150 cases per 100,000 population. The incidence of HIV is still relatively low compared to international figures. There has been wide coverage with BCG vaccination since 1960 in Karachi, Pakistan. However, very little is known regarding the pathophysiology of the disease post BCG vaccination. We have

previously published clinical characteristics and drug resistance patterns in this area (Hussain *et al*, 1996). In this report we describe cellular immune responses in patients with active tuberculosis and their household contacts.

MATERIALS AND METHODS

Study subjects

Patients with active pulmonary and lymph node tuberculosis presenting at Masoomeen Hospital in Karachi, Pakistan between January 1991 and June 1992 [N = 119; Pulmonary = 95, lymphadenitis (LN) = 23 Endobronchial (EB) = 1] were studied. The clinical characteristics of these patients have been described in detail previously (Hussain *et al*, 1996). The diagnosis of pulmonary tuberculosis was established by demonstration of acid fast bacilli in sputum, and eventually confirmed by culture of *M.tb.* (Hussain *et al*, 1996). Twenty-three patients had lymph node tuberculosis based on histological findings and on biopsy of their lymph nodes and had no evidence of lung involvement. Patients had received not more than 90 days of antituberculous treatment at the time of study. Subsets of these patients were analysed for various immunological parameters. Healthy endemic controls (N = 51) included employees of The Aga Khan Hospital and were either laboratory workers or faculty. None of the endemic controls had been previously treated for tuberculosis or showed clinical signs of disease. Healthy household contacts (N = 59) were contacts of TB patients with active pulmonary disease. All endemic controls and household contacts had been previously vaccinated with BCG at the time of immunological assessment. Venous blood was obtained with the informed consent of the donor or their parents or guardians as apropriate. PPD (5TU) (Connaught Labs, Ontario, Canada) skin testing was administered to all patients and read at 72 hours by the same individual.

Antigens

Phytohemagglutinin (PHA) and pokeweed mitogen (PWM) were purchased (Sigma Chemicals, USA). Purified protein derivative (PPD) of *M.tb.* were obtained through the courtesy of Dr Jan van Embden (RIVM, The Netherlands). *M. tuberculosis* culture filtrate (*M.tb.* CF) and sonicate (*M.tb.* Son) were a gift from Dr HJ Kolk (The Royal Tropical Institute, The Netherlands). The preparation of these antigens has been described previously (Verbon *et al*, 1990). Recombinant *M. tb.* 71K, *M. tb.* 64K and *M. leprae* 18K were obtained through the courtesy of Dr Jan van Embden (Netherlands) prepared with the support of UNDP/World Bank/WHO Special Program.

Serum

Five milliliters of venous blood was collected from both patients and controls and allowed to clot overnight at 4°C. Serum was removed and centrifuged at 400 *g* for 15 minutes; the clear supernate was distributed in 100µl aliquots and frozen at -70°C until use.

Assay of lymphocyte blastogenesis.

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood (30 ml) by density sedimentation over Ficoll-Hypaque. Cells were washed 3 times with medium (RPMI 1640; BioWhittaker, Walkersville, MD, USA). Cells were counted and suspended in complete medium (RPMI 1640 with 2mM L-glutamine, 100 mg/ml of gentamicin, 15 mM HEPES and 10% pooled human sera). Two hundred thousand cells per well were placed in a round bottom microtiter tissue culture plate (Falcon Products, Becton Dickinson, Oxnard, CA, USA). PHA (1 µg/ml), SLO 1:100, tetanus toxoid 1/50, PPD, *M. tb.* culture filtrate and *M. tb.* sonicate (10 µg/ml), were added to triplicate wells for each variable. These concentrations were found to be optimal for each stimulant after testing a series of concentrations. In control wells cells received medium alone. The cultures were incubated for 5 days in 5% CO_2 at 37°C. One microcurie of ^{3}H-thymidine (specific activity 6.7 curies/mM [Amersham, UK]) was added to each culture well for the final 24 hours. Cells were harvested after 24 hours with a PHD harvester (Cambridge Technology, Cambridge, MA, USA) and ^{3}H-thymidine content was measured in a scintillation counter. Results were expressed as mean counts per minute of the triplicates. A response was considered positive if the counts incorporated in experiments were ≥ 1,500 cpm higher than counts incorporated in cells cultured with medium alone. Spontaneous

incorporation of ^{3}H-thymidine in cultured cells ranged between 500-1,000 counts per minute.

Assay for interferon γ in culture supernatants of stimulated PBMC's

Supernatants were collected from stimulated cells after 5 days and assayed for interferon gamma secretion by ELISA based assays. Interferon gamma standard was a UK reference obtained from the National Institute of Biological Standards (NIBSC, UK). Plates were coated with 100µl containing 2.5 µg/ml of mouse monoclonal anti human interferon γ (Genzyme, Cambridge, MA, USA) in carbonate buffer 0.06M pH 9.6 and incubated overnight at 4°C. The plates were washed and incubated with the reference and test samples for 2 hours at 37°C. The plates were subsequently further incubated (37°C) with rabbit polyclonal antibodies (1/1,000) to interferon γ (gift of Dr J Blackwell, Cambridge, UK). The revealing probe was goat anti-rabbit immunoglobolin conjugated to horse radish peroxidase (Jackson laboratories, Westgrove, PA, USA) and OPD was color used as a substrate for color development. The reaction was stopped with 4M H_2SO_4 and the reaction read at 410 nm in a Titerteck plate reader MR 600 (Dynatech).

Quantitation of polyclonal and *M. tb.* specific IgE antibodies

Quantitation of PC and antigen specific IgE have been described in detail previously (Hussain *et al*, 1995). Plates were coated with purified rabbit anti-human IgE at 2µg/ml. Sera were incubated for 2 hours at 37°C and subsequently overnight at 4°C. Mouse monoclonal antibody HP6029 was added at a 1:1,000 dilution and incubated overnight at 4°C. Goat anti-mouse IgG conjugated to alkaline phosphatase was used as the revealing probe followed by appropriate substrate. Between each step, the plates were washed three times with PBS containing 0.05% Tween 20. An eight-point calibration curve was developed with a standard reference serum (myeloma PS; a kind gift from Dr T Ishizaka) with known amounts of IgE. All test sera were run at a minimum of three serial dilutions. Values falling in the mid-range of the dose response curve were used to calculate the concentration of IgE in test sera.

For determination of IgE anti *M. tb.* culture filtrate (*M. tb.* CF), Immulon 4 plates were coated with 100 µl of *M. tb.* CF at 1µg/ml in carbonate buffer pH 9.6 for 2 hours at 37°C and then overnight at 4°C. PBS containing 5% BSA was added for 2 hours at 37°C to block free sites. 100µl of sera diluted in PBS containing 0.05% Tween 20 and 1.0% BSA was added and incubated for 2 hours at 37°C and then overnight at 4°C. Monoclonal antibodies specific for IgE were added at saturating concentrations of 1:500 (HP 6029), and further incubated overnight at 4°C. Alkaline phosphatase-labeled goat anti-mouse IgG was incubated for 2 hours at 37°C. The plates were finally developed with alkaline phosphatase substrate. Each incubation was followed by three washes with PBS containing 0.05% Tween 20 to remove unbound protein. For IgE antibodies, all sera were run at a dilution of 1:10 in a single assay and the results for these antibodies are expressed as optical density (OD) readings.

Statistics was done on an Apple Macintosh computer using Statview™ software packages. Mann-Whitney analysis was used for comparison of patient and control groups.

RESULTS

Pulmonary disease severity as stratified by age and gender of the patients is depicted in Fig 1. Patients with pulmonary tuberculosis were classified according to the extent of tissue involvement on chest roentgenograms as minimal (PMN), moderate (PMD) or advanced (PAD) disease. The majority of patients in all age groups as well as in both genders showed moderate pulmonary disease. The age distribution among the two genders was different with females showed peak level in the second decade of life and subsequently decreasing with age while males showed equal distribution across all age groups. Immunological studies were carried out in this group of patients.

Tuberculin skin test response

Skin tests showed a singificant negative correlation with age in patients ($N = 88$; $p < 0.03$) using Spearman Rank correlation. The scatter and linear regression are depicted in Fig 2. Table 1 shows the

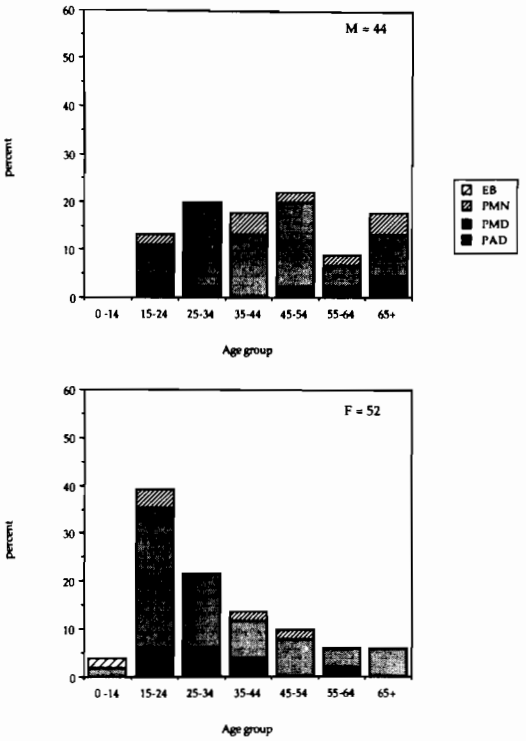

Fig 1–Disease severity in relation to age and gender.

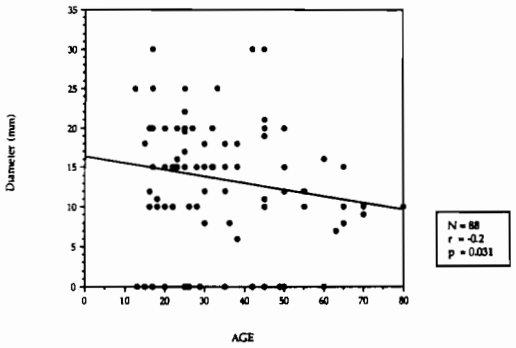

Fig 2–Correlation of skin test reactivity with age. Significance was determined using Spearman rank analysis. Each point represents an individual patient.

percent of skin test positivity (cut off > 10 mm) and mean diameters of induration in relation to disease severity. As the extent of pulmonary disease increased from moderate to advanced, both percent positivity and the mean diameter of positive responses decreased indicating a down regulation of delayed type hypersensitivity (DTH) in advanced pulmonary tuberculosis. Patients with lymph node disease showed the strongest tuberculin skin test response with ulceration in the majority of cases. A high percentage of healthy endemic controls (48%) and household contacts (70%) were also positive. The positivity in household contacts compared to endemic controls is probably indicative of recent exposure while positivity in endemic controls may be due to previous BCG vaccination. However, recent exposure cannot be ruled out in the endemic controls even though there was no history of recent exposure in this group.

Table 1

PPD skin test responses in relationship to disease severity.

Group ID	N	%Pos*	Mean ± SE
TB Pulmonary			
i) PAD	13	62	9.5 ± 2.4
ii) PMD	59	81	14.1 ± 0.9
iii) PMN	6	67	13.8 ± 4.8***
TB lymphadenitis	23	100	20.8 ± 2.8
Household contacts	23	70	12.4 ± 1.6
Endemic controls	24	48	10.8 ± 2.0

* PPD Skin test was considered positive if the diameter of induration at 72 hours was > 10mm

** Mann-Whitney test was carried out to assess differences with endemic controls. All sifnificant values ($p < 0.05$) are underlined.

except*** $p = 0.09$.

Lymphocyte blastogenic responses (LTT)

We assessed the relationship of LTT to PPD and PPD skin tests in 81 patients where paired responses were available (Fig 3). The *in vitro* and *in vivo* tests of the response for PPD showed a highly significant correlation when Spearman rank analysis was carried out ($p < 0.006$).

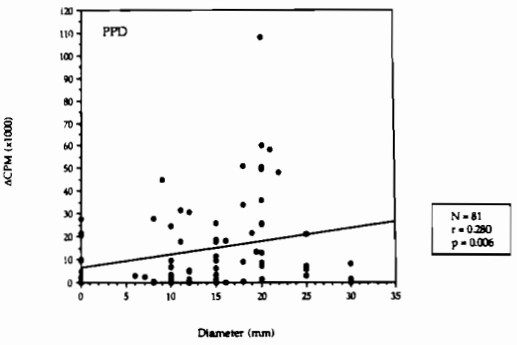

Fig 3–Correlation between skin test and LTT. Significance was determined using Spearman rank analysis.

To compare cellular responses in patients during active disease with healthy endemic controls, LTT responses were assessed to a panel of mitogen/recall antigens as well as mycobacterial specific antigens (Fig 4a, 4b, 4c). Whereas LTT responses to the mitogen PHA were comparable in the patient and control groups, significantly depressed cellular responses were detected to mycobacterial crude ($p < 0.01$) and recombinant antigens ($p < 0.001$) as well as recall antigens SLO and TT ($p < 0.01$). These results suggest an overall suppression of memory T-cell function. LTT responses in patients with respect to disease severity are shown for *M. tb.* sonicate only (Table 2) as responses to all three crude mycobacterial antigens containing slightly different repertoires of antigens was similar. The most depressed responses were observed in patients with the most advanced pulmonary disease. LN group did not show suppression of cellular responses as seen with the pulmonary group. These findings parallel the skin test responses observed in the same patient categories. However, both endemic controls and household contacts showed stronger LTT responses than skin test responses, indicating that in the early phase of the response, cells other than Th1 subset may be proliferating in response to mycobacterial antigens while later in

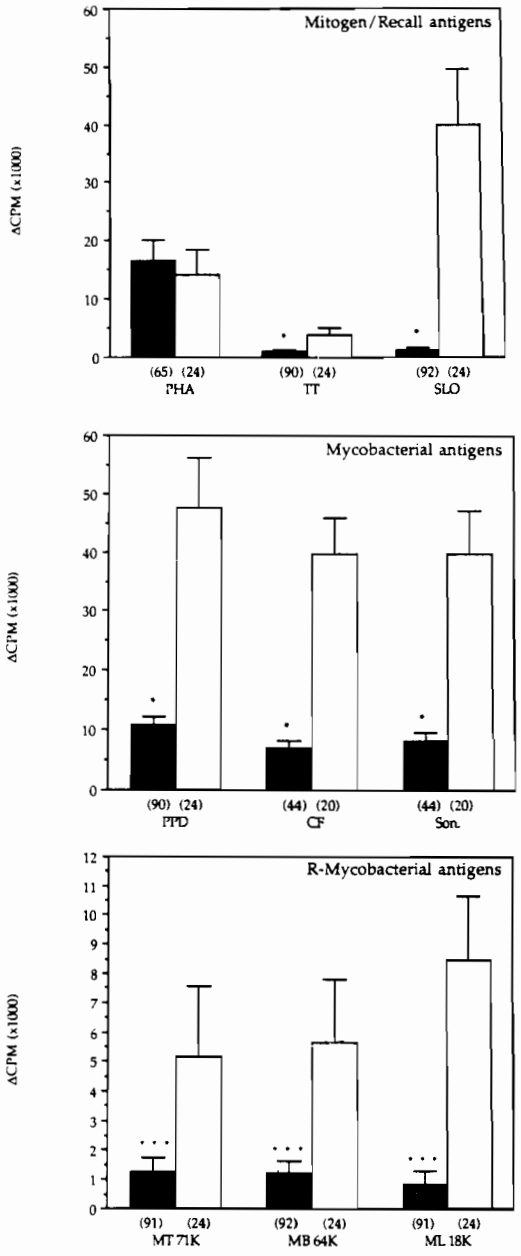

Fig 4a,4b,4c–Comparison of LTT responses in patients and endemic controls. Boxes indicate group mean and the vertical line standard error around the mean. Numbers in brackets show group sizes. Filled boxes are values for patients and open boxes indicate control values. Mann-Whitney analysis was used to determine the significance between the two groups. (*indicates $p < 0.001$).

Table 2

Lymphoproliferative responses in relation to disease severity.

Group ID*	N	LTT (ΔCPM)**
TB pulmonary		
i) PAD	13	9,401 ± 2,439
ii) PMD	59	11,532 ± 2,014
iii) PMN	6	18,917 ± 8,149
TB lymphadenitis	23	49,200 ± 13,947
Household contacts	23	50,473 ± 8,543
Endemic controls	34	47,544 ± 8,758

* Mann-Whitney test was carried out to assess difference with enemic controls. All values with $p < 0.05$ are underlined.

** Lymphocytes were stimulated with 10μg/ml of PPD. Group means are expressed as ΔCPM after deduction of spontaneous incorporation.

the immune response the proliferating population is mainly restricted to Th1 subset.

Interferon γ responses

Since chemotherapy can result in immunological recovery a subset of pulmonary and lymph node patients who had received < 30 days of treatment were included in this group. Also a socio-economically matched group of healthy household contacts of patients with active disease were included for comparison of early immune responses after recent exposure. As expected, the rank order of LTT and interferon gamma responses were the same in the 3 groups (Table 3). Patients with pulmonary disease showed significantly lower responses ($p < 0.05$) for both LTT and interferon γ compared to household contacts. Lymph node patients had slightly higher responses for both LTT and interferon γ but the responses were not significantly different to those of healthy household contacts. It would thus appear that Th1 responses are activated very early after exposure to *M. tb.* and before the establishment of disease. However, it is unclear if these responses are progressively downregulated as the infection establishes itself or if individuals who do not mount Th1 response (14% of HC were skin test negative) are susceptible to disease establishment. This point can only be clarified by carrying out follow up studies on household contacts for development of disease.

Polyclonal and antigen-specific IgE responses

As a surrogate marker of Th2 responses we also determined IgE responses in patients with pulmonary and lymph node tuberculosis and compared them with healthy endemic controls. Significantly raised polyclonal IgE responses were detected in all patient groups as well household contacts. However, antigen specific IgE was significantly raised only in pulmonary patients indicating that sequestration of antigen in the lung but not in the lymph node results in a bias towards Th2 responses.

Table 3

Comparison of LTT and interferon γ in TB patients and controls.

Group ID	N	LTT (ΔCPM)*	Interferon γ**
TB pulmonary	29	14,135 ± 2,831	49.7 ± 8.3
TB lymphadenitis	12	35,129 ± 10,220	115 ± 25.8
Household contacts	29	27,570 ± 3,939	111 ± 11.8

* LTT Lymphocyte transformation test (LTT) is expressed as ΔCPM incorporated after stimulation with 10 μg/ml of *M. tuberculosis* culture filtrate after deduction of spontaneous incorporation.

** Interferon γ released in LTT supernatants is expressed as international units/ml.

Statistical significance determined by Mann-Whitney analysis. Values underlined are significantly lower $p < 0.01$ compared to household contacts.

Table 4

Distribution of polyclonal and *M. tuberculosis* specific IgE antibodies in patients with active tuberculosis.

Group ID	N	PC IgE*	IgE anti CF**
TB pulmonary			
i) PAD	30	2,002 ± 844	0.113 ± 0.015
ii) PMD	105	2,809 ± 932	0.105 ± 0.006
TB lymphadenitis	23	954 ± 625	0.099 ± 0.012
Household contacts	59	1,863 ± 687	0.094 ± 0.006
Endemic controls	51	506 ± 192	0.084 ± 0.006

* Results expressed as ng/ml

** Results expressed as OD @ 1/20 dilution

Student's *t*- test was used to determine the significant differences in antibodies between endemic controls and patient groups. Significantly raised ($p < 0.05$) values are underlined.

DISCUSSION

The nature of the immune response is critical in determining the disease outcome in terms of protection or susceptibility both in experimental animal models and in man. Suppression of Th1 and augmentation of Th2 responses has been associated with disease progression due to several intracellular pathogens (Modlin and Nutman, 1993; Romagnani, 1994; Reiner and Lackey, 1995). Although Th1 responses in tuberculosis have been well documented, Th0 (characterized by production of a mixture of Th1 and Th2 cytokines) rather than Th2 cell responses have been described in tuberculosis at the systemic level (Barnes *et al*, 1993; Del Prete *et al*, 1993). Most of the clinical studies may have been biased by small sample sizes. We have tried to document the dynamic relationship between different arms of the immune response by carrying out concurrent analysis of Th1 (PPD skin test, LTT and interferon γ) and Th2 response (using IgE as a surrogate marker) in patients with active tuberculosis involving sequestration of *M. tuberculosis* in different compartments (lung *vs* lymph node) and household contacts of patients with active tuberculosis. The strength of this study is the dissection of cellular responses in a large series of well-characterized patients using a varied panel of myco-bacterial, recall and recombinant antigens. The clinical and hematological characteristics of this patient group as well as humoral responses have been previously reported (Hussain *et al*, 1995, 1996). We now report the cellular immune responses as assessed by various parameters indicative of Th1 and Th2 activation. Our studies clearly demonstrate that Th1 responses are consistently suppressed only in patients with moderate to advanced pulmonary tuberculosis.

Skin test response which is an indicator of delayed type hypersensitivity (Th1 response) showed a decline with age as well as progressive disease. This is in keeping with the observation that cohorts of individuals show a declining prevalence of tuberculin reactivity with age (Grozbowki *et al*, 1964). Cellular responses are known to decrease with age due to many factors (Hannet *et al*, 1992). Thus PPD skin test anergy may be contributed both by age and disease status of the patients and the effect of both variables should be kept in mind while interpreting PPD skin tests in tuberculosis patients. One dilemma that arises due to introduction of BCG vaccination is the usefulness of PPD skin test as a diagnostic marker in tuberculosis disease endemic areas. Our results show a high rate of positivity in healthy endemic controls, and decreased positivity in moderate to advanced disease. Both variables limit the usefulness of PPD skin tests as a diagnostic marker of tuberculosis. Interestingly, there was no effect of age on severity of disease since all age groups showed a similar spectrum of lung involvement. The highest percentage of patients in all age group showed moderate pulmonary disease. This was not because of early reporting at our clinic which ranged between 1-3 months from the start of

symptomatology; this is similar to the reporting period in Cleveland, Ohio where a much higher percentage of advanced disease is seen (unpublished observations). One possibility is that the decreased severity may be a consequence of BCG vaccination which may confer partial immunity as the majority of our patients had been vaccinated previously. Another possibility is modulation and downregulation of immune responses in disease endemic areas which is a well documented observation for several parasitic infections in expatriates such as the Peace Corps Volunteers from USA who developed much more severe disease in African countries than the indigenous population. However, for tuberculosis this has not been documented so far. This issue can only be resolved by assessing disease severity in BCG vaccinated areas where the clinical spectrum of disease severity is known prevaccination.

As an *in vitro* correlate of delayed type hypersensitivity, lymphocyte blastogenesis was assessed in response to several mycobacterial antigens. There was excellent correlation between tuberculin skin test reactivity and PPD-stimulated blastogenesis in patients with tuberculosis. Overall suppression of T-cell responses to *M. tb.* antigens was observed in patients with active pulmonary disease as compared to healthy endemic controls and extended to other recall antigens; the level of suppression increased with increasing lung involvement. These observations parallel our PPD skin test observations in the same group of patients and support the correlation observed between LTT and PPD skin tests. Protective responses are associated with activation of mononuclear phagocytes by Th1 cytokines particularly interferon γ which appears to be central to anti-mycobacterial responses. To address the issue of interferon γ production in early infection *vs* progressive disease, we studied a group of newly diagnosed pulmonary patients (treated for >30 days with moderate to advanced disease) and their household contacts who were living in the same environment, and therefore were actively exposed and were derived from the same socio-economic environment. Again, interferon γ was lower in patients with active pulmonary disease than in household contacts who were exposed but had no clinical signs of the disease. Patients with lymph node disease showed concentrations similar to household contacts. Thus sequestration of *M. tb.* in the lymph node compartment results in strong Th1 responses. Similarly other studies have shown that in patients with tuberculous pleuritis, where sequestration of *M. tb.* occurs in another compartment, the pleural space and tissue surrounding the lungs also have a highly antigen-responsive and Th1 cytokine producing cell population compartmentalized to the pleural space (Ellner, 1978a; Fujiwara *et al*, 1982; Barnes *et al*, 1989). Barnes *et al* (1989) have found high levels of Th1 cytokines interferon γ and TNF α in the pleural fluid. Analysis of the pleural fluid mononuclear cells by PCR indicated selective concentrations of mRNAs encoding interferon γ and IL10 in pleural fluid compared with PBMCs of the same patients (Barnes *et al*, 1990) strongly supporting our conclusion that antigen specific suppression is consistently present in pulmonary patients and is not a general feature of all clinical presentations of tuberculosis.

The observed suppression of Th1 responses in pulmonary disease was not related to the absence of responsive T-cells as PHA responses were comparable in both the control and patient groups. However, the observed suppression of cellular responses may be related to depressed PPD-stimulated expression of IL-2 in far-advanced pulmonary tuberculosis (Toossi *et al*, 1985). Depressed PPD-stimulated blastogenesis in patients with pulmonary tuberculosis appears due, at least in part, to a suppressive influence of monocytes (Ellner *et al*, 1978b). The mechanism of suppression involves direct stimulation by PPD (Wallis *et al*, 1986) of monocytes primed during the course of tuberculosis to produce suppressive predictors such as IL-2R (Toossi *et al*, 1990) and transforming growth factor-beta (Toossi *et al*, 1995; Hirsch *et al*, 1996). CD16 lymphocytes also suppress PPD-induced responses (Toossi *et al*, 1989). The antigenic-specificity of suppression through the monocyte depends on direct stimulation by PPD and would not account for decreased response to non-mycobacterial antigens as noted in this and some other (Ellner, 1978), but not all (Toossi *et al*, 1985) studies. Sequestration or localization of *M. tb.* in different compartments results in varying intensity of Th1 activation and the ontogeny as well as the milieu of other cytokine circuits activated (Toossi, 1996), rather than the intensity of Th1 responses may be critical to disease establishment.

These suppressive pathways seem to abate during treatment of tuberculosis (Toossi *et al*, 1989). Therefore we studied a subset of pulmonary patients who had received $\leq$ 30 days of anti-tubercu-

lous chemotherapy for both lymphoproliferative and interferon γ studies so that the immune profile would not be influenced by chemotherapy. The profile of lymphocyte proliferative responses in this subset (Table 3) was similar to that observed for the larger panel with significant suppression of LTT and interferon γ responses in patients with pulmonary disease. This may be due the fact that the majority (> 65%) of our patient group were newly diagnosed patients (≤ 30 days of chemotherapy). We are further investigating the effect of varying duration of chemotherapy on different immune parameters.

With respect to Th2 responses, our results clearly show that as the pulmonary disease advances antigen specific IgE responses rise. Patients with lymph node disease who demonstrate the strongest Th1 responses do not show significantly raised antigen-specific IgE responses. Interestingly, patients with lymph node disease did show slightly elevated polyclonal IgE responses which were not statistically significant. This may be due to either non-specific polyclonal activation which is known to occur during active tuberculosis or may indicate increased incidence of parasitic infections in this group. This is supported by our observation that polyclonal IgE was also raised in household contacts derived from the same socio-economic strata. Elevated Th2 responses associated with depressed Th1 responses in pulmonary patients are in keeping with the cross regulation of Th1/Th2 responses reported in the murine model (Bottomly, 1988). Furthermore, different antigen presenting cells in different compartments may also play a role in activation of different T cell subsets. Thus alveolar macrophages may be effective in activating Th2 cells while dendritic cells in the lymph node compartment shift the response towards a Th1 response. Further dissection of local and systemic immune responses at the cytokine level may identify additional differences between lymphadenitis and pulmonary disease. It is of interest to speculate that some of the mediators ("cross-modulatory cytokines") overexpressed by activated monocytes in tuberculosis could themselves contribute to the increased antibody levels by promoting isotype switching and post-switch differentiation of B-cells. We have previously reported polyclonal activation of all isotypes but only selective increases in antibody IgG subclasses which was restricted to IgG1 and IgG3 in pulmonary patients compared to the control group and were highest in patients with extensive disease (Hussain *et al*, 1995). The relationship between cytokines, cellular and antibody responses needs to be further clarified in tuberculosis in order to understand disease pathogenesis. We are now investigating the nature of cellular and humoral responses in disseminated disease where there is no lung involvement, to identify surrogate markers for disease progression and pathogenesis.

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REFERENCES

Barnes PF, Abrams JS, Seiling PA, *et al.* Patterns of cytokine production by mycobacterium-reactive human T-cell clones. *Infect Immun* 1993; 61 : 197-203.

Barnes PF, Fong SJ, Brennan PJ, *et al.* Local production of tumor necrosis factors and interferon-γ in tuberculous pleuritis. *J Immunol* 1990; 145 : 149-54.

Barnes PF, Mistry SK, Cooper CL, *et al.* Compartmentalization of a CD4+T-lymphocytes subpopulation in tuberculous pleuritis. *J Immunol* 1989; 142 : 114-9.

Bottomly K. A functional dichotomy in CD4+ T-lymphocytes. *Immunol Today* 1988; 9 : 268-74.

Del Prete G, De Carli M, Almerigogna F, *et al.* Human IL10 is produced by both Type 1 helper (Th1) and Type 2 helper (Th2) T cell clones and inhibits their antigen specific proliferation and cytokine production. *J Immunol* 1993; 150 : 353-60.

Ellner JJ. Pleural fluid and peripheral blood lymphocytes function in tuberculosis. *Ann Intern Med* 1978a; 89 : 932-3.

Ellner JJ. Suppressor adherent cells in human tuberculosis. *J Immunol* 1978b; 121 : 2573-8.

Fine PEM. BCG vaccination against tuberculosis and leprosy. *Br Med Bull* 1988; 44 : 69-703.

Fujiwara H, Okuda Y, Fukakawa T, *et al.* *In vitro* tuberculin reactivity of lymphocytes from patients with tuberculous pleurisy. *Infect Immunol* 1982; 35 : 402-9.

Grozbowki S, Allen EA. The challenge of tuberculosis in decline. A study based on epidemiology of tuberculosis in Ontario, Canada. *Am Rev Respir Dis* 1964; 90 : 707-20.

Hannet I, Erkeller-Yuksel F, Lydyard P, *et al.* Developmental and maturational changes in human blood lymphocytes subpopulations. *Immunol Today.* 1992; 13 : 215-8.

Hirsch CS, Hussain R, Dawood G, *et al.* Cross modulatory role for transforming growth factor-β in tuberculosis; suppression of antigen-driven interferon-gamma production. *Proc Natl Acad Sci USA* 1996; 93 : 3193-8.

Hussain R, Dawood G, Abrar N, *et al.* Selective increases in antibody isotypes and IgG subclass responses to secreted antigens in tuberculosis patients and household contacts. *J Lab Diag Immunol* 1995; 2 : 726-32.

Hussain R, Hasan R, Khurshid M, *et al.* Pulmonary tuberculosis in a BCG vaccinated area: relationship of disease severity with immunological and hematological parameters and drug resistance patterns. *Southeast Asian J Trop Med Public Health* 1996; 27 : 257-62.

Modlin RL, Nutman TB. Type 2 cytokines and negative immune regulation in human infections. *Curr Opin Immunol* 1993; 5 : 511-7.

Reiner SL, Lockley RM. The regulation of inmmunity to Leishmania major. *Ann Rev Immunol* 1995; 13 : 151-77.

Romagnani S. Lymphokine production by human T cells in disease states. *Ann Rev Immunol* 1994; 12 : 227-57.

Toossi Z. Cytokine circuits in tuberculosis. *Infect Agents Dis* 1996; 5 : 98-107.

Toossi Z, Edmonds KL, Tomford WJ, *et al.* Suppression of PPD-induced interleukin-2 production by interaction of Leu-22 (CD16) lymphocytes and adherent mononuclear cells in tuberculosis. *J Infect Dis* 1989; 159 : 352-6.

Toossi Z, Gogate P, Schiratsuchi H, *et al.* Enhanced production of transforming growth factor-β (TGF-β) by blood monocytes from patients with active tuberculosis and presence of TGF-β in tuberculous granulomatous lesions. *J Immunol* 1995; 154 : 465-473.

Toossi Z, Kleinhenz ME, Ellner JJ. Defective interleukin-2 production and responsiveness in human pulmonary tuberculosis. *J Exp Med* 1985; 163 : 1162-72.

Toossi Z, Lapurga JP, Ondash RJ, *et al.* Expression of functional IL-2 receptors by peripheral blood monocytes from patients with active pulmonary tuberculosis. *J Clin Invest* 1990; 85 : 1777-84.

Verbon A, Kuijper S, Jansen HM, *et al.* Antigens in culture supernatant of *Mycobacterium tuberculosis*: epitopes defined by monoclonal and human antibodies. *J Clin Microbiol* 1990; 136 : 955-64.

Wallis RS, Fujiwara H, Ellner JJ. Direct stimulation of monocyte release of interleukin-1 by mycobacterial protein antigens. *J Immunol* 1986; 136 : 193-6.