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T4 lymphopenia in patients with active pulmonary tuberculosis

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SUMMARY

The numbers of cells bearing the T3 (pan-T cell), the T4 (putative helper/inducer cells), the T8 (putative suppressor/cytotoxic cells) and B cell phenotypic markers were counted in venous blood samples from 26 newly diagnosed pulmonary tuberculosis patients and 29 healthy controls from East Java. The absolute T cell count was lower in the patients and T4 cells were fewer in patients (mean 748/mm^3) than in controls (mean 1,043/mm^3), but there were no significant differences in total T8 cell and B cell counts between patients and controls. The T4:T8 ratio was not disturbed in many patients, but it was less than 1·6 in 11 of 26 patients and in only three of 29 controls: this ratio was less than 1·2 (the lower limit of 'normal') in six patients but no controls. The intensity of the T4 lymphopenia was unrelated to the extent of the lesion seen radiologically or the size of the skin test reaction to PPD. Levels of interferon-α were not elevated in the serum of any of the patients or controls. It is suggested that the T4 lymphopenia was a reaction to the mycobacterial infection and not a manifestation of underlying secondary (acquired) immune deficiency.

Keywords T4 lymphopenia tuberculosis

INTRODUCTION

Virulent strains of *Mycobacterium tuberculosis* are highly infectious for normal individuals who usually develop cutaneous sensitivity to products of the bacillus 4–8 weeks after infection (Wallgren, 1948). Only a small proportion of these individuals (less than 10%) go on to develop overt disease (Styblo, 1980), presumably because of the considerable efficiency of natural protective immunity. The pathogenesis of tuberculosis is complicated and the factors that predispose to, and determine the outcome of, this and other mycobacterial diseases are still poorly understood and largely anecdotal (Fine, 1984). While some patients have obvious natural or iatrogenic causes of immunosuppression that predispose to disease, they form a small minority. Various forms of immunosuppression are found in patients with tuberculosis (Rook, 1983), but these appear to be the result rather than the cause of the disease. From the clinical point of view it would be very useful to be able to assess the protective immune reactivity of a patient with tuberculosis, and the effect of therapy on such responses, but at present this is not practicable. One indirect approach to this

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problem would be to use monoclonal antibodies (MoAb) to enumerate the lymphocyte subsets since the T8 phenotype marker identifies a subpopulation containing the suppressor cells, while cells bearing the T4 marker are predominantly involved in the protective cell-mediated immune responses.

In a study of acquired immune deficiency in 10 previously healthy Haitians, Vieira *et al.* (1983) noted that five of the patients had been treated for tuberculosis with rifampicin before the onset of opportunistic superinfection. They stated that it was possible, but highly unlikely, that tuberculosis, or its treatment with isoniazid or rifampicin, could have altered the relative numbers of circulating lymphocytes bearing the cell membrane markers T4 (putative 'helper/inducer' cells) and T8 (putative 'suppressor/cytotoxic' cells), but they dismissed this possibility because of the severity of the altered T4:T8 ratio (similar in extent to that seen in the acquired immune deficiency syndrome, AIDS) and the absence of AIDS associated opportunistic infection in classical tuberculosis.

A MEDLARS search (June 1984) has however shown that there have not been any published reports of counts of lymphocytes bearing T4 and T8 phenotypic markers in the peripheral blood of pulmonary tuberculosis patients. We now report relatively frequent occurrence of moderate T4 lymphopenia in patients with untreated, but otherwise uncomplicated pulmonary tuberculosis. Since more severe T4 lymphopenia is a prominent feature of AIDS where opportunistic infection is common and serum interferon-α (IFN-α) levels are usually high, IFN-α levels were measured in the tuberculosis patients and found to lie within normal limits.

SUBJECTS AND METHODS

The study was made on 26 patients (20 men and six women; aged 17–67 years) at the time of radiological confirmation of the diagnosis of pulmonary tuberculosis just before the start of anti-tuberculous drug treatment and on 29 control subjects (18 men and 11 women; aged 19–60 years) with no radiological abnormality in the chest from the same socio-economic group as the patients. All subjects were citizens of Surabaya or adjacent parts of East Java (Indonesia). Skin tests with 5 TU of PPD (RT23, Batch No. 83225, Biofarma, Bandung, Indonesia) had been performed 2 days previously and this was read at the time of venepuncture. A white blood cell count (WBC) was made and the percentage of lymphocytes in a differential count of a blood film was recorded. Twenty millilitres of blood were collected in heparinized tubes and held at ambient temperature (30–32°C) for 2–3 h during transport to the laboratory. The mononuclear cells were separated by density gradient centrifugation over Ficoll-Hypaque (Sigma Chemical Co., Poole, UK) (Böyum, 1968), washed three times with Hank's balanced salt solution (BSS) (Gibco, Paisley, UK) and recovered by centrifugation at 75*g* for 10 min at a laboratory temperature of 20°C. All subsequent manipulations for MoAb staining were performed at 4°C. One million cells in 200 μl of the standard TC medium (RPMI 1640, Gibco, containing 5% fetal calf serum, FCS) were incubated with 5 μl MoAb for 30 min. Leu 1 was used as a pan-T marker, Leu 3 for T4 cells, Leu 2a for T8 cells and Leu 12 for B cells (all from Becton-Dickinson, Twickenham, UK): all were diluted for use according to the manufacturer's recommendations. Thereafter the cells were washed twice in TC medium with 5% FCS and bound mouse Ig was visualised by staining with FITC conjugated sheep anti-mouse Ig (Serotec, Bicester, UK) for 30 min. The unattached conjugate was removed by washing twice in TC medium. The proportion of stained lymphocytes was counted in wet preparations under transmitted light dark ground illumination with blue–violet light from a high pressure mercury vapour lamp (HBO 200): with ×100 oil immersion objective, viable lymphocytes could be distinguished from neutrophil granulocytes (PMN), monocytes and dead or damaged lymphocytes on the basis of the dark ground image. The absolute number of T cells, T4 and T8 cells and B cells in the peripheral blood were then calculated from knowledge of the total lymphocyte count.

The concentration of INF-α in sera was measured immunoradiometrically with the Sucrosep kit (Boots-Celltech Diagnostics Ltd., Slough, UK). All serum specimens had been stored in the laboratory at −80°C and transported to the UK at −70°C. Each specimen was assayed in duplicate along with appropriate positive and negative control sera, which gave anticipated results.

RESULTS

After density gradient centrifugation over Ficoll-Hypaque the layer of cells at the interface with the supernatant plasma formed a compact whitish–grey band with all the samples from control Indonesian subjects, similar to our previous experience with healthy European subjects. However, with many of the samples from pulmonary tuberculosis patients the bands were broader, less distinct and extended into the upper zone of the Ficoll-Hypaque cushion: sometimes it was double layered and on other samples it was very abundant and contaminated with small aggregates of erythrocytes which had failed to fall to the bottom of the tube. Microscopic examination of IF stained preparations confirmed that the mononuclear cell fraction from blood samples of patients were often heavily contaminated with PMN, whereas the fractions from the control Indonesian subjects had little PMN contamination and were in all respects comparable to our previous experience on healthy European subjects.

The total lymphocyte count in the peripheral blood was somewhat lower in the pulmonary tuberculosis patients (mean 1,519/mm^3, s.d. 409) than in the controls (mean 1,713/mm^3, s.d. 662), but this difference was not significant. The absolute T cell count was also lower in the patients (mean 1,221/mm^3, s.d. 337) than in the controls (mean 1,549/mm^3, s.d. 522) and this difference was significant ($t = 2{\cdot}659$; $P < 0{\cdot}005$). The absolute T4 and T8 cell counts are shown in Fig. 1. T4 cells are less numerous in pulmonary tuberculosis patients (mean 748/mm^3, s.d. 62) than in controls (mean, 1,043/mm^3, s.d. 86) and this difference was highly significant ($t = 11{\cdot}61$, $P < 0{\cdot}001$): nevertheless many patients have an absolute T4 count within the lower part of the normal range. By contrast the number of circulating T8 cells was similar in patients (mean/487 mm^3, s.d. 36) and controls (mean 465/mm^3, s.d. 50). The T4:T8 ratios are shown in Fig. 2: these are significantly lower ($t = 3{\cdot}062$, $P < 0{\cdot}0025$) in the patients (mean 1·71, s.d. 0·64) than in the controls (mean 2·25, s.d. 0·61). A T4:T8 ratio was not disturbed in many patients, but it was less than 1·6 in 11 of 26 patients and in only three of 29 controls: this ratio was less than 1·2 (the lower limit of 'normal') in six patients and no controls. T4 lymphopenia and T4:T8 ratios less than 1·6 were not related to the apparent clinical or radiological severity of the disease or particular types of lesion seen on radiological examination. Absolute T4 and T8 cell counts were unrelated to the mean diameter of induration in the Mantoux

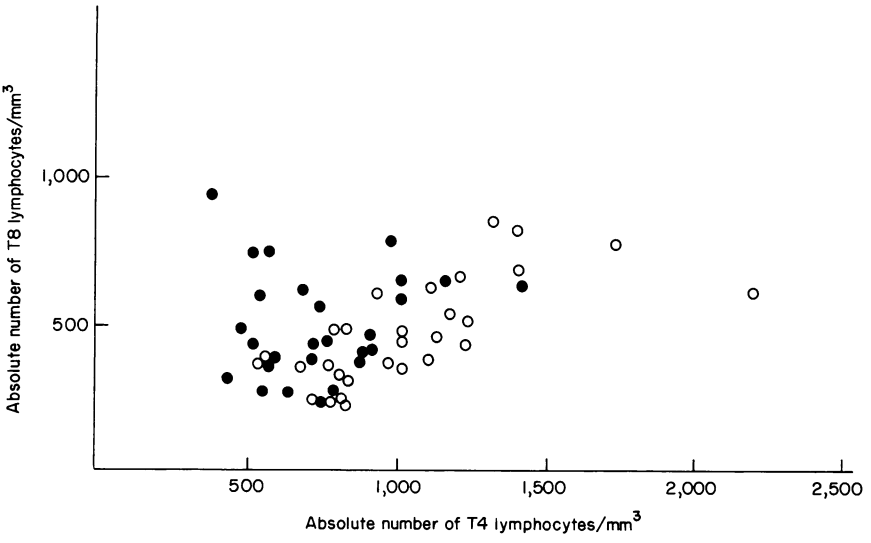

Fig. 1. Absolute counts of lymphocytes bearing T4 and T8 phenotypic markers in mononuclear cell fractions from venous blood of 26 pulmonary tuberculosis patients (●) and 29 healthy controls (○). There is no significant difference in T8 cell counts between the two groups, but T4 cells are significantly less numerous in pulmonary tuberculosis patients ($P < 0{\cdot}001$).

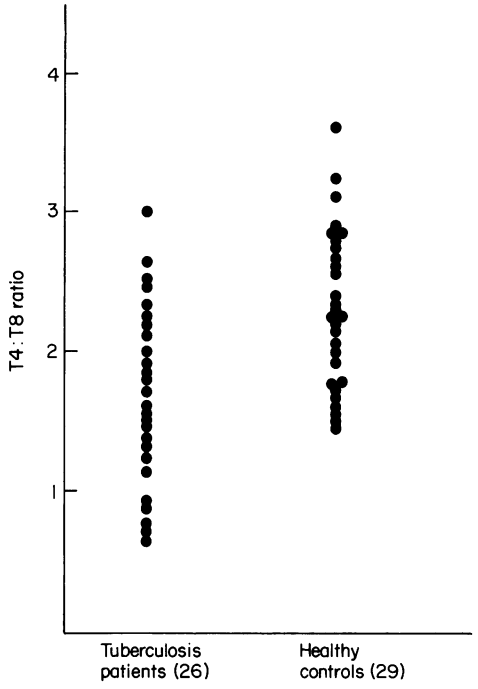

Fig. 2. T4:T8 ratios in 26 pulmonary tuberculosis patients and 29 healthy controls: 11 of the patients have values less than 1·6 compared with only three of the controls.

reaction measured at 48 h (for both $r < 0{\cdot}1$). No significant difference was seen between the patients and the controls in absolute B cell count or in the ratio of the absolute counts of T and B cells.

The concentration of IFN-α in the sera of 26 patients and 24 controls was in all cases less than 1 u/ml.

DISCUSSION

The mononuclear cell concentrate obtained by density gradient centrifugation of blood from healthy donors over Ficoll-Hypaque usually has little contaminaton with PMN or RBC, but the method often yields contaminated fractions from blood samples for ill patients (Aiuti *et al.*, 1975; Currie *et al.*, 1978; Gibbs *et al.*, 1982). Such heavy PMN contamination was a noticeable feature of samples from patients with active untreated pulmonary tuberculosis, but it was not seen in samples from healthy Javanese controls or treated leprosy patients studied concurrently in the laboratory: it is perhaps noteworthy that the leprosy patients were better nourished than the tuberculosis patients and apparently in a better physical state. The enumeration of lymphocyte subsets was made in this investigation by direct microscopy under dark ground illumination, since this technique ensured confident distinction between lymphocyte and monocytes, PMN and RBC: the counts of MoAb stained lymphocytes were meaningful, although tedious to perform.

The counts of absolute numbers of lymphocytes in the T4 and T8 subsets and the calculated T4:T8 ratios that we found in the healthy Indonesian controls were similar to our previous experience in healthy Europeans: others have reported similar figures in USA (Fahey *et al.*, 1984). There is only one previous report of counts of T cell subsets in Indonesian subjects (Hoffman *et al.*, 1984): the values for total T cells and T4 bearing cells in their two control groups were generally similar to those in the present paper, but they found much higher numbers of T8 cells (mean 705 and 996/mm^3) than in the present study (465/mm^3). Perhaps part of the discrepancy can be explained by

the need for Hoffman *et al.* (1984) to transport the blood samples long distances to the laboratory in Jakarta involving air journeys up to 10 h: overnight storage at 4°C diminishes the T4:T8 ratio (Dzik & Neckers, 1983; Murray & Rahl, 1983) and reduces the response to the PHA stimulation (Feldmeier & Kerr, 1982). We conclude that the T4 lymphopenia we observed is a genuine alteration associated with active untreated pulmonary tuberculosis.

Moderate reduction in the T4:T8 ratio has been observed previously in lepromatous leprosy (Mshana *et al.*, 1982; Wallach, Cottenot & Bach, 1982) and this reverts to normal under effective chemotherapy (Mshana, Haregewoin & Behehu, 1982), but these papers do not state whether the imbalance was due to reduction in numbers of T4 cells or to increase in T8 cells. Nevertheless it is tempting to speculate that the changes are analogous to those we now report in tuberculosis and that they are a consequence of the ongoing immune response in the disease, since a relative T4 lymphopenia has been reported in normal subjects after booster immunization with tetanus toxoid (Eibl, Mannhalter & Zlabinger, 1984) and as part of an immunologically specific response after antigenic bronchial provocation in asthmatics (Gerblich, Campbell & Schugler, 1984). Interestingly, comparable T4 lymphopenia has been reported in haemophiliacs treated with Factor VIII from a population apparently free from AIDS and this change has been attributed to a reaction to transfusion of foreign proteins (Carr *et al.*, 1984). It is very unlikely that the skin test with PPD 48 hr before venepuncture induced the T4 lymphopenia in the tuberculosis patients, since the healthy controls were unaffected despite often having cutaneous reactions of comparable size and moreover, the extent of T4 lymphopenia was unrelated to the size of the indurative reaction in the skin test. Perhaps T4 cells have accumulated preferentially in the tuberculous lesions, but this possibility has not yet been investigated. Another possible explanation is that the T4 cells become sequestered in lymph nodes since in experimental mycobacterial infections in mice (Rook, 1976) recirculating T cells are trapped in lymph nodes. Selective accumulation of antigen specific T cells in lymphoid tissue has been observed in Ugandan patients with advanced tuberculosis, particularly those who are skin test negative (Rook, Carswell & Stanford, 1976). It is possible that such trapping mechanisms affect the T4 cells to a greater extent than the T8 cells.

T4 lymphopenia of much greater severity than that seen in the present investigation has gained widespread recognition as a prominent feature of AIDS (Gottlieb *et al.*, 1981; Schroff *et al.*, 1981) and the persistent generalized lymphadenopathy syndrome in homosexual men (? AIDS) (Mathur-Wagh *et al.*, 1984). In AIDS patients IFN-α is very commonly raised (>8 u/ml) (Preble, Eyster & Goedert, 1982). We have therefore looked for this abnormality in our patients with recently diagnosed active tuberculosis: all of the patients had levels of IFN-α <1 u/ml, suggesting that the modest T4 lymphopenia in tuberculosis is a reaction to the mycobacterial infection and not an indication of underlying immunosuppression of the type seen in AIDS, but the long term implications of T4 lymphopenia in our patients remains to be established.

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