ORIGINAL ARTICLE

γδ T lymphocytes in the peripheral blood of patients with tuberculosis with and without HIV co-infection

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Thorax 2002;**57**:357–360

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Revised version received 6 September 2001
Accepted for publication 31 October 2001

Background: Several recent studies suggest that γδ T lymphocytes play an important role in immunity against *Mycobacterium tuberculosis*. However, the dynamics of these cells in the peripheral blood of patients with tuberculosis (TB) with and without HIV infection is not fully understood. A study was undertaken to evaluate the profile of the γδ T cell population in patients at the time the diagnosis of TB was established.

Methods: A cross sectional study was performed in consecutive TB patients from the Department of Infectious Diseases, Spedali Civili, Brescia. CD4+, CD8+ and Vδ1 and Vδ2 T cell counts were analysed. Lymphocyte surface membrane expression was evaluated with the FITC-TCRγδ, -Vδ1, -Vδ2 and PE-Vδ1 monoclonal antibodies. Blood donors and HIV seropositive asymptomatic individuals acted as controls.

Results: Seventy four TB patients were evaluated, 20 of whom (27%) were co-infected with HIV. HIV seronegative TB patients (n=54) had total γδ T cells and Vδ1 subsets comparable to those in blood donors (n=39). However, the percentage with the Vδ2 subset was significantly lower in patients with TB than in controls (median 1.5 *v* 2.1; p=0.05). Responsiveness to PPD was not associated with predominance of a specific γδ T cell subset. HIV seropositive individuals had a decreased percentage of circulating Vδ2 cells at a level similar to that in HIV seronegative TB patients, regardless of the presence of active TB.

Conclusions: HIV seronegative TB patients and HIV infected individuals (with or without active TB) have a reduced number of circulating Vδ2 T cells compared with healthy individuals. Whether TB and HIV infection share a common mechanism causing Vδ2 T cell depletion still needs to be established.

The role of γδ T lymphocytes in the immune response to *Mycobacterium tuberculosis* has been the object of several recent studies. Activation of both Vδ1 and Vδ2 cells during tuberculosis (TB) infection has been shown in vivo, but in vitro studies with mycobacterial antigens have shown that the enhanced response is mainly due to the Vδ2 subset.[1] The magnitude of this activation varies according to the phase of TB infection (acute or chronic) and to the immune status of the host, from Vγ9/Vδ2 cell proliferation to anergisation.[2] In HIV infected individuals a reduced prevalence of one γδ T cell subset has been reported; this consists mainly of an inversion in the proportion of Vδ1/Vδ2 cells with a selective increase in the Vδ1 subset.[3] An in vitro associated lack of responsiveness of Vδ2 cells to mycobacterial antigens has also been described.[4 5]

This study was performed to evaluate the profile of γδ T lymphocytes in the peripheral blood of both HIV infected and non-infected patients at the moment the diagnosis of TB is established.

METHODS

Patients and controls

A cross sectional study was performed involving consecutive TB patients who sought care at the Department of Infectious Diseases, Spedali Civili from January 1998 to June 2000. The diagnosis of TB was based on the presence of a positive culture of *M tuberculosis* from a clinical specimen together with systemic symptoms and signs of disease. After giving written informed consent, patients not known to be HIV seropositive were tested for the presence of HIV-1 antibodies by enzyme immunoassay (Murex Diagnostics, Norcross, USA) confirmed by Western blotting (Abbott Laboratories, North Chicago, USA). A tuberculin skin test was carried out using 5 TU purified protein derivative (PPD) intradermally on the volar surface of the forearm and the transverse induration was measured 48–72 hours later. Indurations of ⩾5 mm and ⩾10 mm, respectively, for HIV seropositive and HIV seronegative subjects were considered as positive responses. Randomly selected blood donors (all HIV seronegative) and asymptomatic HIV seropositive individuals (matched with TB-HIV co-infected patients for clinical stage, CD4 cell count and HIV-RNA plasma levels) were used as controls.

Lymphocyte surface phenotype analysis

For γδ T cell analysis 10 ml venous peripheral blood was taken before beginning antituberculous treatment. Evaluation of lymphocyte surface membrane expression was performed on whole blood samples with fluorescein isothiocyanate conjugated (FITC) monoclonal antibodies anti-TCRγδ, -Vδ1, and -Vδ2 (Endogen, Woburn, MA, USA) and Tetrachrome CD45/CD4/CD8/CD3 (Coulter Immunology, Hialeah, FL, USA). Samples were analysed by flow cytometry (Epics XL; Coulter).

Statistical analysis

Microsoft Access 2000 was used for data collection and management and SPSS 7.0 for Windows was used for statistical analyses. Univariate analyses of the association of sociodemographic and clinical categorical variables with the HIV serostatus of TB patients were performed using a χ^2 test. The Mann-Whitney U test was used to compare the proportions of total lymphocytes, CD4+, CD8+, γδ T cells and subsets between patients and control groups. Overall differences in the percentage of γδ T lymphocytes in all four study groups were evaluated using the Kruskal-Wallis test. The chosen level of significance was 5%. The p values reported are two tailed.

Table 1 Demographic and clinical features of HIV infected and non-infected patients with TB

Characteristics	All patients (n=74)	TB HIV+ (n=20)	TB HIV– (n=54)	p value†
Median (range) age (years)	32 (2–78)	35 (6–44)	31 (2–78)	0.67
Male (%)	54 (73)	15 (75)	39 (72)	1.0
Foreign born (%)	55 (74)	10 (50)	45 (83)	0.006
PPD positivity (%)	34 (72)*	11 (79)	23 (70)	0.73
Pulmonary TB (%)	47 (63.5)	16 (80)	31 (57)	0.10

*n=47; † χ^2 test except for comparison of ages (Mann-Whitney U test).

Table 2 Median (range) percentage of total lymphocytes, CD4+, CD8+, γδ T cells, and Vδ1 and Vδ2 subsets among TB patients and controls

Cell population	TB HIV+ (n=20)	HIV+ asymptomatic (n=9)	p value*	TB HIV– (n=54)	Blood donors (n=39)	p value†
Total lymphocytes	22.0 (4.6–77.0)	20.4 (6.8–68.0)	0.85	23.8 (5.4–65.0)	34.5 (24.8–47.9)‡	<0.0005
CD4+	15.0 (1.2–38.0)	12.6 (2.6–38.7)	0.73	43.1 (3.4–57.3)	46.1 (36.9–60.9)	0.05
CD8+	56.6 (33.7–72.3)	62.1 (4.1–78.8)	0.5	24.5 (6.1–68.2)	24.5 (14.5–37.7)	0.92
Total γδ T cells	4.2 (2.0–12.3)	3.7 (1.8–9.8)	0.32	3.35 (0.7–11.9)	4.0 (0.8–15.3)	0.16
Vδ1	1.75 (0.9–11.3)	1.8 (0.9–6.3)	0.83	1.0 (0.1–6.3)	0.9 (0.1–6.8)	0.75
Vδ2	1.05 (0.3–3.9)	0.9 (0.3–3.7)	0.34	1.5 (0.2–11.8)	2.1 (0.5–13.4)	0.05

*Mann-Whitney U test comparing HIV+TB patients and HIV+ asymptomatic patients; †Mann-Whitney U test comparing HIV– TB patients and blood donors; ‡n=23 for total lymphocytes, CD4+ and CD8+ cell counts.

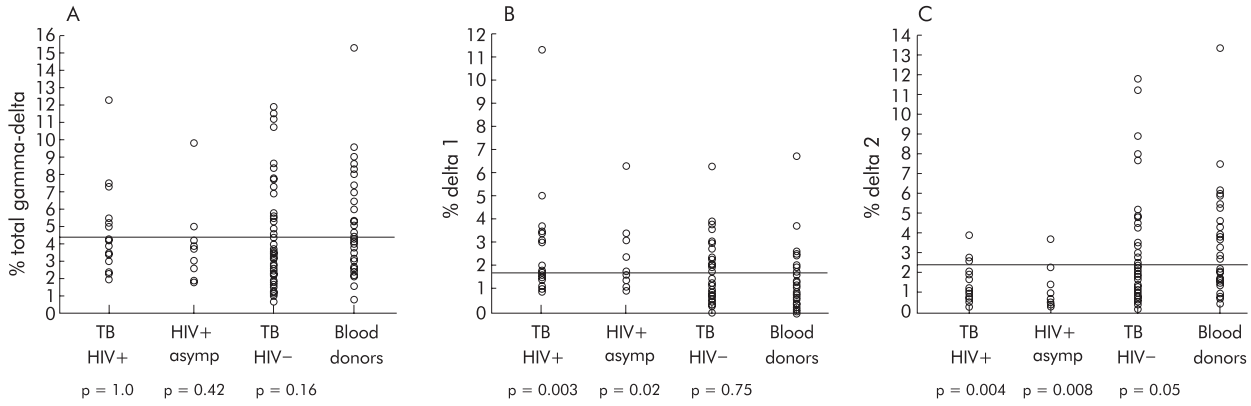

Figure 1 Percentage of (A) total γδ T lymphocytes, (B) Vδ1 and (C) Vδ2 subpopulations in the peripheral blood of patients with TB and controls. p values (Mann-Witney U test) indicate the significance of the difference in distribution compared with healthy blood donors. Horizontal lines represent the mean values of the entire population.

RESULTS

During the study period 74 patients with TB were evaluated, 20 (27%) of whom were co-infected with HIV. There were 54 (73%) men, the median age was 32 years, and 55 (74%) were foreign born patients. Foreign born patients represented a significantly higher proportion of HIV negative patients (45/54; 83.3%) than HIV positive patients (10/20; 50.0%; p=0.006). Pulmonary TB was the most common clinical presentation (63.5%). In the HIV seropositive TB patients the median CD4+ cell count was 268 cells/mm^3 (range 15–623); seven (35%) had values of ≤200 /mm^3. Table 1 summarises the demographic and clinical characteristics of the study patients.

The relative proportions (median percentage) of total lymphocytes, CD4+, CD8+ and γδ T cells with Vδ1 and Vδ2 subsets of HIV seronegative and seropositive TB patients were compared with those of 39 healthy blood donors and nine HIV seropositive asymptomatic patients (table 2). HIV seronegative TB patients had lower total lymphocyte and CD4+ proportions than healthy blood donors but similar total γδ T cell and Vδ1 subset proportions. However, a statistically significant reduction in circulating Vδ2 T cells was observed in HIV negative TB patients (1.5) compared with healthy blood donors (2.1, p=0.05).

The percentage of total γδ T cells and Vδ1 and Vδ2 subsets was similar in HIV positive TB patients and HIV positive asymptomatic patients. Both groups had higher proportions of Vδ1 and lower proportions of Vδ2 subsets than healthy blood donors (p≤0.02). There was a statistically significant overall difference in the percentages of Vδ1 and Vδ2 subpopulations between all groups (p=0.002 and p=0.02, respectively) that was not observed for total γδ T cells (p=0.60). Figure 1 represents the distribution of γδ T cells in TB patients and controls.

As expected, there was a lower proportion of CD4+ T lymphocytes and a higher proportion of CD8+ T lymphocytes in HIV seropositive than in HIV seronegative TB patients. Moreover, HIV seropositive TB patients had a similar proportion of Vδ2 subsets as HIV seronegative TB patients, but a statistically significant increase in the proportion of Vδ1 T cells (p=0.25 and p=0.004, respectively, table 2). Similarly, there was no difference in the proportion of Vδ2 subsets between asymptomatic HIV seropositive and HIV seronegative TB patients (p=0.10), but the former had a higher proportion of Vδ1 T cells (p=0.02, table 2).

Patients with TB with a negative PPD skin test had a lower total lymphocyte count than those with a positive skin test. However, when the γδ T proportions were compared, no

Table 3 Median (range) percentage of total lymphocytes, CD4+, CD8+, γδ T cells and δ1 and δ2 subsets in TB patients according to response to PPD skin test

Cell population	PPD+ TB patients (n=34)	PPD– TB patients (n=13)	p value*
Total lymphocytes	27.1 (4.8–65.0)	17.8 (8.9–39.9)	0.02
CD4+	37.9 (5.8–56.9)	33.5 (1.2–56.5)	0.16
CD8+	29.1 (15.0–72.3)	25.8 (6.1–72.0)	0.78
Total γδ T cells	3.4 (1.0–12.3)	4.3 (1.2–11.9)	0.25
Vδ1	1.4 (0.1–11.3)	1.2 (0.4–6.3)	0.90
Vδ2	1.3 (0.4–11.2)	1.8 (0.3–11.8)	0.69

*Mann-Whitney U test.

significant difference in the γδ T cell proportions was seen between PPD positive and PPD negative TB patients (table 3). When only HIV seronegative TB patients were analysed, no significant differences in γδ T cell proportions associated with different PPD responses were observed ($p>0.05$).

DISCUSSION

The aim of this study was to evaluate the population of γδ T lymphocytes in the peripheral blood of TB patients in an attempt to offer new insights into the role played by these cells in immunity against *M tuberculosis*. TB patients, independent of HIV serological status, had a reduced proportion of circulating Vδ2 subsets compared with healthy controls. This observation confirms and expands the findings of Li and coworkers who reported a significant reduction in the proportion of the Vδ2 subset among circulating γδ T cells of TB patients, postulating a quantitative reduction of this subpopulation.[6] Other studies which have found no variations in the γδ T cells of TB patients have reported on total γδ T cells but did not measure the Vδ2 subset.[7 8] All these data are consistent with our findings: it is the Vδ2 subpopulation which is specifically affected, but it does not result in significant changes in the proportion of total γδ T cells. Our data differ from those of a recent report by Dieli and coworkers who analysed the whole γδ T cell population and its δ2 subset and reported similar proportions in PPD positive children with TB and in healthy PPD positive and PPD negative children.[9] These data, however, were obtained in a paediatric population and biological differences between children and adults may account for the difference in the results.

On the other hand, some studies have reported an increase in the proportion of γδ T cells in the peripheral blood of TB patients. However, in one study the percentage of γδ T cells was in the normal range reported in the literature while the comparison groups had abnormally low levels.[10] In another study the sample size was small, with few cases with abnormally high levels of γδ T cells.[11]

The interaction of γδ T cells and *M tuberculosis* seems to vary according to the phase of TB infection. Newly infected individuals show an increase in γδ T cells that is mainly of the Vδ2 phenotype. Ueta and coworkers described a higher percentage of γδ T lymphocytes associated with the presence of activation markers (suggesting antigen driven amplification) in healthy hospital professionals recently exposed to patients with TB.[12] It has been proposed that, after the initial increase in γδ T cells that occurs during the primary infection, these cells return to normal levels during chronic TB infection.[2] The observation of a reduction in the Vδ2 population at the time active TB is diagnosed is consistent with the suggested role of these cells in maintaining equilibrium between the host and *M tuberculosis* during chronic infection.

No significant differences in γδ T cell proportions were seen in PPD positive and PPD negative TB patients. The relationship between γδ T lymphocytes and the tuberculin skin response is not clear. Barnes found a greater response of the γδ T population to mycobacterial antigens in healthy PPD positive individuals and patients with pleuritis than in those with pulmonary or miliary tuberculosis, supporting the hypothesis that the increase in the γδ T cell population could be associated with protective immunity.[8]

The level of circulating Vδ2 T cells in HIV seropositive individuals, regardless of the presence of active TB disease, was similar to that in TB patients without HIV infection. All HIV seropositive subjects had a non-specific increase in the Vδ1 cell subset, a decrease in the Vδ2 subset, and an inversion of the Vδ1/Vδ2 proportions. These results agree with those described by other authors who have consistently reported inverted Vδ1/Vδ2 proportions with an increase in the Vδ1 cell population.[3 13 14] The reduction in Vδ2 cells observed in HIV seropositive subjects has been attributed to the presence of specific ligands inducing a sustained activation of Vδ2 cells, followed by a reduction in this cell subset by spontaneous and activation induced apoptosis.[5 15] Whether the non-specific decrease in Vδ2 T cells in HIV seropositive subjects has an adjunctive role to CD4+ T lymphocyte dysfunction in the increased susceptibility of HIV infected subjects to TB disease is unclear. HIV and TB infection could carry or induce common ligands resulting in a reduction rather than an increase in the Vδ2 T cell population, but this hypothesis still needs to be tested.

Our study could not establish whether the reduction in the Vδ2 subset of γδ T cells is a predisposing factor to the development of TB or is a consequence of mycobacterial infection itself. Ellner described the presence of *M tuberculosis* specific suppressor monocytes in some TB patients that could more selectively inhibit antigen induced proliferation of γδ T cells.[16] The sequestration of reactive γδ T lymphocytes in the TB site of disease is another possible explanation for the reduction in the Vδ2 subpopulation in the peripheral blood of patients with TB.[17]

We did not measure the γδ T cell response to mycobacterial antigens nor the presence of activation markers in the peripheral blood of TB patients. We therefore cannot affirm that the quantitative changes seen in γδ T cells correspond to functional derangement of this lymphocyte population. Further studies in a larger number of patients, with follow up evaluations and qualitative analysis of the γδ T cell population in response to mycobacterial infection, could contribute to our understanding of the function of this lymphocyte subset in immunity against *M tuberculosis*.

ACKNOWLEDGEMENT

This study received financial support from III Programma Nazionale di Ricerca sull'AIDS, Istituto Superiore di Sanità, Rome, Italy (contract 50B.6).

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