

Gamma/delta T lymphocytes in the blood of patients with sarcoidosis

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Abstract

Background – Gamma/delta T lymphocytes are thought to have a role in granulomatous immune responses at peripheral sites of antigen contact such as the gut, skin and lung. The aim of this study was to determine if gamma/delta T lymphocytes are increased in the peripheral blood of patients with active sarcoidosis.

Methods – Peripheral blood from 21 untreated patients with a new presentation of sarcoidosis (12M, 9F), 20 normal volunteers (12M, 8F), and 12 patients with cavitary pulmonary tuberculosis were subjected to Ficoll Hypaque separation and flow cytometry analysis using monoclonal antibodies to CD3, 4, 8, 25, HLA-DR and gamma/delta T cell receptor.

Results – All patients with sarcoidosis had compatible chest radiographs and all were Mantoux negative in spite of previous BCG vaccination. In all but one patient histological examination showed non-caseating granuloma. There was no difference in the mean percentage or absolute numbers of gamma/delta positive peripheral blood lymphocytes between the three populations. Thirteen patients with sarcoidosis had an absolute lymphopenia and the mean percentage of CD3 positive peripheral blood lymphocytes in the group with sarcoidosis was lower than the other two groups. The percentage of CD25 and HLA-DR positive cells was higher in the group with sarcoidosis, supporting the fact that these patients had active disease.

Conclusion – Gamma/delta T lymphocytes are not increased in the peripheral blood of patients with sarcoidosis and are unlikely to have a role in the pathogenesis of this disease.

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Keywords: T lymphocytes, sarcoidosis, gamma/delta cells.

Sarcoidosis is a multisystem disorder of unknown aetiology characterised by granuloma formation at the sites of disease activity.¹ It is likely that the granuloma formation is due to antigen specific lymphocyte involvement. There is evidence that lung T cells in patients with sarcoidosis have increased T cell receptor (TCR) V beta mRNA transcripts and preferentially use specific TCR antigen receptor elements suggesting that accumulated T cells represent a response to persistent specific antigen stimulation.²⁻⁴ Most T cells recognise antigen through the T cell antigen receptor $\alpha\beta$

CD3 complex on the T cell surface.⁵ A small percentage of T cells, however, express a second type of TCR complex designated gamma/delta ($\gamma\delta$). Peripheral blood T cells expressing $\gamma\delta$ TCR have been identified primarily at epithelial surfaces such as the skin, gut, and lung. This suggests that the cells may represent the first line of immune defence as they are found at the sites of initial antigen contact.⁶⁻⁹ Gamma/delta cells have been shown to recognise certain mycobacterial antigens including heat shock proteins in vitro.^{10,11} In addition they have been shown to accumulate in the granulomatous skin lesions of patients with leprosy and cutaneous leishmaniasis. When cultured from leprosy lesions $\gamma\delta$ cells proliferate in response to mycobacterial antigens and secrete various cytokines.¹² More recently it has been suggested that $\gamma\delta$ cells are increased in the peripheral blood of patients with tuberculosis.¹³ Current evidence suggests therefore that $\gamma\delta$ cells with specificity for foreign antigen may be important in the pathogenesis of granulomatous disorders. In view of the evidence for a possible specific antigen as an aetiological agent in the pathogenesis of sarcoidosis and the predominant localisation of disease activity at sites of presumptive antigen exposure (lung, skin, eyes, and lymph node), we asked whether $\gamma\delta$ T lymphocytes might be important in the pathogenesis of this disease.

Methods

Blood samples were taken by venepuncture from adult patients with biopsy proven sarcoidosis presenting for the first time or with a recurrence of previously documented sarcoidosis. Patients were excluded from the study if they had a positive Mantoux test, a history of tuberculous infection or disease, if they had received corticosteroid therapy within the previous six months, or if they had another intercurrent illness or infection. Appropriate cultures were performed to exclude active tuberculosis (bronchoalveolar lavage fluid, sputum, urine) at the time of presentation and no patients have subsequently developed tuberculosis.

Twenty one patients were recruited into the study (see results for clinical characteristics), 12 with active pulmonary tuberculosis and 20 healthy adults served as controls. Ethical committee approval was obtained.

Twenty ml of blood was subjected to Ficoll Hypaque separation and the resultant mononuclear cell population was washed then incubated with autologous serum for 15 minutes. The cells were then washed and incubated for 30 minutes with Fitc and/or PE conjugated

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Table 1 General characteristics of the populations studied

	Sarcoidosis (n = 21)	Normal subjects (n = 20)	Tuberculosis (n = 12)
Age	31 (21–44) (12 M, 9 F)	32 (22–44) (12 M, 8 F)	48 (17–79) (7 M, 5 F)
Chest radiograph			
Stage I	12	ND	NA
Stage II	3	ND	NA
Stage III	6	ND	NA
Erythema nodosum	6	0	0
Other symptoms	11	0	12
Peripheral blood lymphopenia	13	0	3
Negative Mantoux	21	ND	2
Positive tuberculosis culture	0	ND	11

monoclonal antibodies to the following antigens CD3, CD4, CD8, CD25, HLA-DR (all Dako), alpha/beta (WT31 recognising a non-polymorphic determinant of the $\alpha\beta$ TCR and hence all $\alpha\beta$ cells), and $\gamma\delta$ (TCR delta 1 recognising the delta protein expressed in association with the gamma chain and hence all $\gamma\delta$ positive cells) [both Becton Dickinson]. Controls included cells alone with no antibody or cells incubated with isotype matched PE or Fitc conjugated mouse antibody. After washing the cells were analysed immediately using a fluorescence activated cell sorter (FACS 440 Becton Dickinson with an argon laser 488 nm). The proportion of positive cells was calculated by subtracting the background control values.

Lymphocytes were discriminated from blood monocytes based on the characteristic low forward angle and 90 degree light scatter profiles on FACS analysis. For two colour immunofluorescence studies the percentage of cells staining positive for each antibody was calculated by setting four quadrants with double staining cells in the upper right quadrant. This was performed on 11 of the 21 patients with sarcoidosis. For the purposes of the analysis $\gamma\delta$ cells were identified as: TCR delta 1 positive, CD3 positive, CD4 negative, CD8 negative.

The absolute number of cells was calculated using the total white blood cell count and cell differential, and the percentage of cells was determined by FACS analysis.

DATA ANALYSIS

The data were expressed as median plus range as stated in text and figures. A mean value is occasionally quoted. The Kruskal-Wallis one way analysis of variance was used to compare results between the three groups. Where data have been compared between two groups the Mann-Whitney U test was used. A probability of <0.05 was considered significant.

Results

STUDY POPULATIONS

Twenty one patients with sarcoidosis were enrolled (12 men, mean age 31 (21–44) years, table 1). The diagnosis was established using previously described criteria and all but one patient had a confirmed histological diagnosis (transbronchial biopsy, lymph node biopsy). The remaining patient had classical Heerfordts syndrome (otherwise known as uveoparotid fever, a syndrome characterised as in this case by parotid gland enlargement, facial nerve

palsy, and submandibular lymphadenopathy). Three patients were smokers. No patient was on treatment at the time of investigation or within the previous six months. All patients were Mantoux negative, 20 of the 21 having previously received BCG vaccination. The median bronchoalveolar lavage lymphocyte count (%) was 35 (5–75). The chest radiograph was abnormal in all cases: grade I, 12; grade II, 3; grade III, 6. Six patients had extrapulmonary disease (cardiac (1), skin (1), spleen (1), eyes (2), granulomatous hepatitis (1)) and six presented with erythema nodosum. Nine patients had new respiratory symptoms (cough and/or dyspnoea) and many complained of profound fatigue (n = 11). All patients were seen within one month of developing these symptoms. The mean ESR was 25 (2–126). Two patients had hypercalcaemia.

Twelve patients (seven men) of mean (range) age 48 (17–79) years with pulmonary tuberculosis were studied. Seven were smear positive with cavitory disease, four were culture positive from either sputum samples or bronchial washings. The remaining patient, a contact of two immediate family members with culture positive disease, had a positive Mantoux test and pulmonary cavities that healed with anti-tuberculous treatment. She was considered to have pulmonary tuberculosis even though she was smear and culture negative for *Mycobacterium tuberculosis*. All patients with tuberculosis had an abnormal chest radiograph, two were Mantoux negative, seven were current or ex-smokers. All patients were HIV negative on serological testing or had no risk factors for HIV infection. Although all patients were receiving antimycobacterial treatment at the time of the study, none had been on treatment for more than one week.

Of the 20 normal controls studied (12 men, mean (range) age 32 (22–44) years), two were current smokers. None had any history of lung disease, tuberculous contact or recent systemic

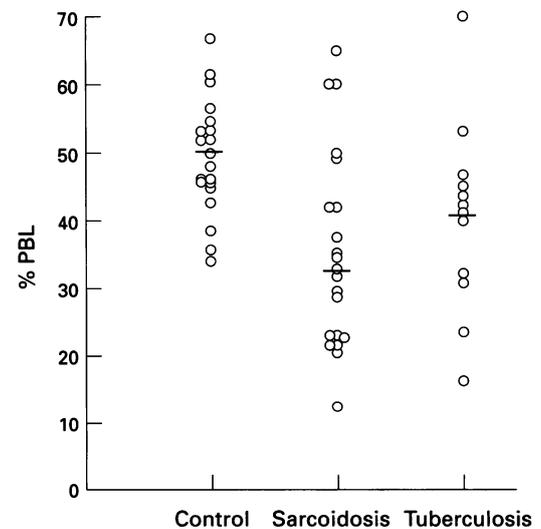


Figure 1 Percentage of CD3 positive cells in the blood. Patients with sarcoidosis had a lower percentage of peripheral blood lymphocytes (PBL) expressing CD3 than controls or patients with tuberculosis ($p < 0.005$, Kruskal-Wallis). The horizontal bar in each group represents the median.

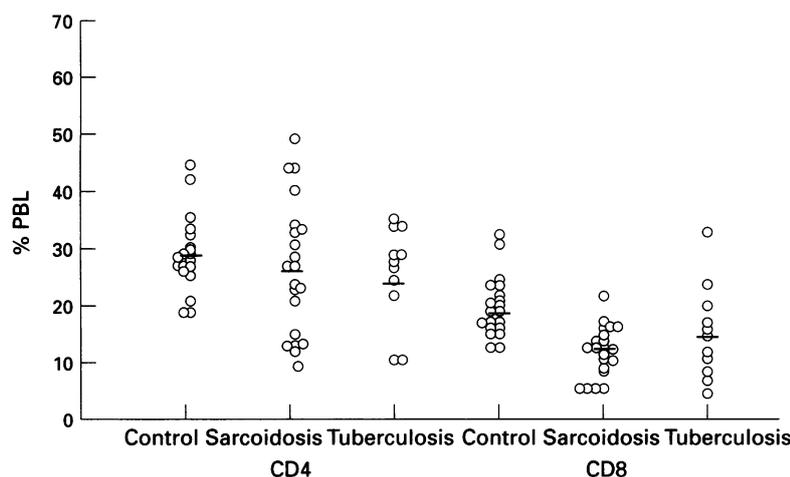


Figure 2 Percentage of CD4 and CD8 positive cells in the blood. Patients with sarcoidosis had a similar percentage of peripheral blood lymphocytes (PBL) expressing CD4 to controls or patients with tuberculosis. There was, however, a lower percentage of CD8 positive peripheral blood lymphocytes in the sarcoidosis group than in controls or patients with tuberculosis ($p=0.001$, Kruskal-Wallis). The horizontal bar in each group represents the median.

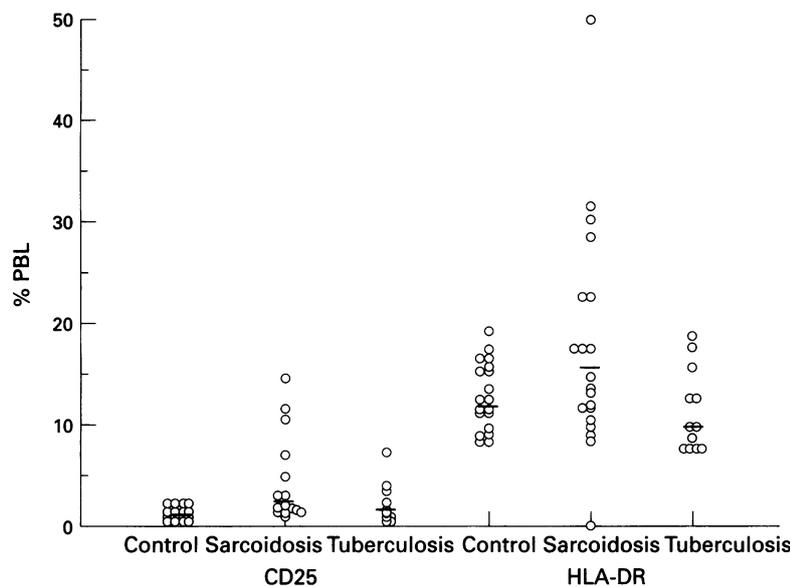


Figure 3 Percentage of CD25 and HLA-DR positive cells in the blood. Patients with sarcoidosis had significantly higher percentages of peripheral blood lymphocytes (PBL) expressing CD25 ($p=0.001$) and HLA-DR ($p<0.05$) than controls or patients with tuberculosis. The horizontal bar in each group represents the median.

illness. Mantoux testing was not performed because the majority had received prior BCG vaccination.

PERCENTAGE CELL COUNTS

The patients with sarcoidosis had a lower percentage of CD3 positive cells than controls or tuberculosis patients ($p<0.005$, Kruskal-

Table 2 Mean (range) absolute numbers of $\gamma\delta$ T lymphocytes/ μ l in the peripheral blood of patients with sarcoidosis and tuberculosis

	n	Total lymphocytes	CD3 +ve lymphocytes	Gamma/delta +ve lymphocytes
Sarcoidosis patients	21	1540 (810-3190)	491 (100-1914)	61 (6-187)
Tuberculosis patients	12	1970 (700-3500)	766 (291-1323)	40 (4-117)
Normal range	20	(1500-4000)	(514-2668)	(2-244)

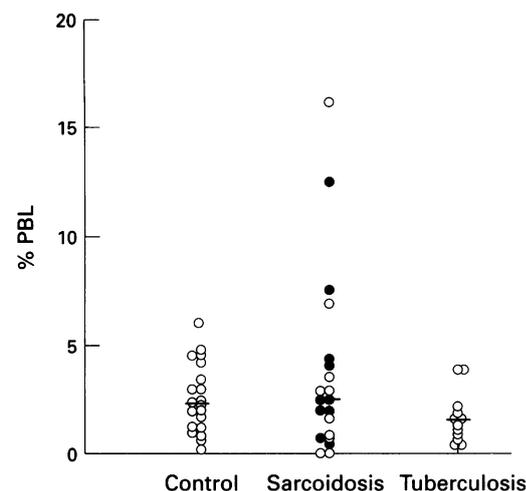


Figure 4 The percentage of $\gamma\delta$ positive cells in the blood. There was no difference between the three groups with respect to the percentage of $\gamma\delta$ cells. The horizontal bar in each group represents the median and the closed circles in the sarcoidosis group represent those samples on which two colour immunofluorescence was performed.

Wallis, fig 1). There was no difference between the groups regarding the percentage of CD4 positive cells but the patients with sarcoidosis tended to have a lower percentage of cells expressing CD8 ($p<0.001$, Kruskal-Wallis, fig 2). Higher percentages of cells expressing CD25 and HLA-DR were found in the patients with sarcoidosis ($p=0.001$, $p<0.05$, Kruskal-Wallis, fig 3).

Although there was no difference between the groups, two patients with sarcoidosis had higher percentages of $\gamma\delta$ T lymphocytes ($>10\%$ peripheral blood lymphocytes) (fig 4). Data (not shown) from the $\alpha\beta$ monoclonal antibody labelling experiments were highly variable.

ABSOLUTE CELL COUNTS

Compared with the other groups the patients with sarcoidosis had a significantly lower mean number of peripheral blood lymphocytes (1540 cells/ μ l, range 810-3910, control range 1500-4000) and 13 of these patients had an absolute lymphopenia (<1500 cells/ μ l) (table 2). The absolute number of CD3 positive cells for the patients with sarcoidosis was low (mean 491, range 100-1914, control range 514-2668). The patients with tuberculosis also had a lower number of CD3 positive cells in their blood (mean 766, range 291-1323), but not to the same extent as the sarcoidosis group.

However, the absolute number of $\gamma\delta$ T lymphocytes for the sarcoidosis group was well within the control range (mean 61, range 6-187 cells/ μ l, control range 2-244). Likewise, there was no increase in the absolute number of $\gamma\delta$ T lymphocytes in the tuberculosis group (mean 40, range 4-117), and no difference between the sarcoidosis and tuberculosis groups ($p=NS$, Mann-Whitney).

Discussion

We have shown in this study that patients with sarcoidosis do not have increased numbers of

$\gamma\delta$ cells in their blood, although an increased percentage of these cells (>10% peripheral blood lymphocytes) was seen in two patients. Both patients had a peripheral blood lymphopenia but not a rise in absolute numbers of $\gamma\delta$ cells, so we believe the elevated percentage of $\gamma\delta$ cells reflects their $\alpha\beta$ lymphopenia. There were no clinical features that distinguished these two patients from the rest of the group. These results are entirely in accordance with earlier published reports.¹⁴⁻¹⁶

We have taken great care in this study to exclude any possibility that our patients with sarcoidosis had tuberculous infection, the presence of which might confound our results. In the event only one patient was excluded because of a positive Mantoux test. None of our patients with tuberculosis had increased proportions or absolute numbers of $\gamma\delta$ cells in their blood. All of these patients had newly diagnosed disease and, with the exception of one, were smear or culture positive. These results are in accordance with those of Tazi *et al*¹⁴ and Barnes *et al*,¹⁷ who likewise found no increase in $\gamma\delta$ cells in the blood of patients with pulmonary tuberculosis. Balbi *et al* found increased proportions and absolute numbers of $\gamma\delta$ cells in only four of 15 patients with tuberculosis.¹⁶ The study by Ito *et al* which did find an increase in $\gamma\delta$ cells in pulmonary tuberculosis is at odds with our report. That study examined 20 patients, but of these the diagnosis was presumptive in seven, and it is not clear how long the patients had been on chemotherapy prior to samples being taken. Although the authors reported an increase in the proportion of $\gamma\delta$ cells in the blood, absolute numbers of these cells were not quantified. The technique for labelling the cells was also different from ours, although the same $\gamma\delta$ monoclonal antibody was used.

With considerable published evidence pointing to the fact that $\gamma\delta$ cells react to mycobacterial antigens *in vitro*, we were somewhat surprised at our own lack of finding $\gamma\delta$ cells in the blood of newly diagnosed patients with pulmonary tuberculosis. It is possible that our failure to detect an increase in blood $\gamma\delta$ cells in either patients with sarcoidosis or tuberculosis is dependent on timing. A number of studies have shown that $\gamma\delta$ cells respond to mycobacterial antigens *in vitro* and kinetics suggest that an early appearing population of $\gamma\delta$ cells represents an appropriate host response to an infecting intracellular organism.^{12,18,19} All of our patients with sarcoidosis had clinical samples taken within 28 days of the onset of symptoms, but it is impossible to be precise about the onset of this disease in these patients. However, our patients did have features which suggest active disease – for example, anergy to PPD, peripheral blood lymphopenia, increased T lymphocyte counts in the bronchoalveolar fluid, and increased percentages of peripheral blood lymphocytes expressing CD25 and HLA-DR. The patients with tuberculosis, the majority of whom had extensive cavitory disease, almost certainly had been infected weeks, if not months, before the study. Thus, our failure to detect $\gamma\delta$ cells in these patients may

reflect our evaluation of chronically diseased patients.

Given that $\gamma\delta$ cells accumulate in the granulomatous lesions of patients with leprosy and leishmaniasis, it is possible that the failure to detect increased numbers of $\gamma\delta$ cells in our patients simply reflects the possibility that we were looking in the wrong place. Sarcoid granulomas are most commonly found in the lungs, lymph nodes, skin, and conjunctiva, and it could be argued that $\gamma\delta$ cells will accumulate at these sites rather than in the peripheral blood. However, Tazi *et al* in an earlier paper have reported that $\gamma\delta$ cells do not accumulate in lymph nodes or Kveim biopsies from patients with sarcoidosis, nor do they accumulate in lymph nodes from patients with tuberculosis.²⁰ Our own provisional results also suggest that these cells do not accumulate in mediastinal lymph nodes, lung biopsy specimens, or bronchoalveolar lavage from patients with sarcoidosis.²¹

In conclusion, we have not found evidence of increased numbers of cells in a well defined population of patients with newly diagnosed sarcoidosis. Given that other studies have also failed to show a consistent increase in $\gamma\delta$ cells in this disease, we conclude that $\gamma\delta$ cells are unlikely to play a part in the pathogenesis of sarcoidosis.

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