γ/δ T lymphocytes in *Mycobacterium tuberculosis* infection

Zoltán Balikó, László Szereday, Júlia Szekeres-Bartho

Abstract

***Background* – Data on the percentage of γ/δ T lymphocytes in the peripheral blood of patients infected with *Mycobacterium tuberculosis* are few and contradictory. The percentage of γ/δ T lymphocytes in the peripheral blood of tuberculin positive and tuberculin negative patients with *Mycobacterium tuberculosis* infection and healthy controls was compared.**

***Methods* – Thirty six patients infected with *Mycobacterium tuberculosis* and 11 healthy controls were studied. Lymphocytes were separated, cytocentrifuged onto glass microscope slides, and reacted with anti-γ/δ monoclonal antibody. The percentage of γ/δ positive cells was determined by microscopic counting of 300 lymphocytes.**

***Results* – No difference was found in the percentage of γ/δ T lymphocytes between patients and controls. However, when the patients were divided into two groups according to reactivity or non-reactivity in the Mantoux skin reaction a higher percentage of γ/δ T lymphocytes was found in the peripheral blood of patients with tuberculin anergy than in tuberculin positive patients or controls.**

***Conclusions* – Higher γ/δ T cell counts are found in tuberculin negative patients with tuberculosis than in tuberculin positive patients or tuberculin positive controls. The high γ/δ T cell counts in tuberculin anergic patients may reflect a shift in the immune response in a Th2 direction characterised by increased antibody production and decreased cell mediated responses.**

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Keywords: γ/δ T lymphocytes, *Mycobacterium tuberculosis*, tuberculin anergy.

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Tuberculosis is the leading cause of death due to a single infectious agent in adults. In the last 10 years the incidence of *Mycobacterium tuberculosis* infection has increased slightly even in the developed countries and there has been a considerable increase in the number of infections caused by multidrug resistant *M tuberculosis*.[1] The development of novel antimycobacterial treatments and more effective vaccines may offer better therapeutic approaches. In order to develop more effective vaccines we have to know more about protective immunity to *M tuberculosis* infection.

Most T lymphocyte receptors consist of alpha and beta chains, and about 10% of the peripheral blood T lymphocytes possess receptors containing gamma and delta chains.[2] The exact role of this population of T lymphocytes is not yet clear.[3] There have been reports of an increased percentage of γ/δ T lymphocytes in the peripheral blood of patients with *M tuberculosis* infection,[4 5] though others have found no difference between patients and healthy controls.[6–8]

The aim of this study was to measure the percentage of γ/δ T lymphocytes in the peripheral blood of patients with tuberculosis who had a positive or negative tuberculin reaction.

Methods

Thirty six patients (23 men) of mean age 46.6 years (range 20–74) infected with *M tuberculosis* were included in the study. Twenty four had a positive sputum culture for *M tuberculosis*, 10 of whom were also sputum smear positive. Patients with negative sputum culture were identified by the typical chest radiographic features and the course of the disease during antituberculous therapy. Tuberculin testing was performed according to the original Mantoux test – that is, 5 TU PPD (Human Rt, Gödöllö, Hungary) were given intradermally in the forearm and the results were evaluated 72 hours later. The skin test was considered positive if there was an induration of 10 mm or more and negative if there was no reaction. Positive reactions of more than 10 mm in diameter were seen in 19 cases (more than 15 mm in six cases) and 17 patients had no tuberculin skin reaction at all. Eleven nurses who had worked in our department for longer than six months (10 women) of mean age 34.2 years (range 19–49) acted as healthy controls. Each had been vaccinated with BCG as part of the required Hungarian national vaccination programme and all were tuberculin positive.

γ/δ T cell counts

Ten ml of venous blood was taken before starting antimycobacterial treatment. Lymphocytes were separated from heparinised venous blood on a Ficoll-Hypaque gradient. The purity of the isolated population was periodically checked by reactivity to anti-CD3 antibody and was found to be consistent. The cells were washed in medium RPMI 1640 (Gibco); the cell count was adjusted to 1×10^6/ml and 100 µl of this suspension was centrifuged and transferred to microscope slides. After air drying the cells

Table 1 Details of patients and controls

	Men	Women	Mean age (range)	Tuberculin skin test		Sputum M tuberculosis	
				Negative	Positive	Smear positive	Culture positive
Patients	23	13	46.6 years (20–74)	17	19	10	24
Healthy volunteers	1	10	34.2 years (19–49)		11		

were fixed with acetone at 4°C for 10 minutes and the cells were reacted with a pan anti-γ/δ monoclonal antibody (T Cells, Sciences, Cambridge, Massachusetts, USA) in a dilution of 1:50. The reacting cells were identified by peroxidase labelled antimouse IgG (Dako) in a dilution of 1:100 using aminoethyl carbasol as chromogen. The percentage of γ/δ T cells was determined by microscopic counting of 300 lymphocytes. Counting was done blind. The patients were divided into two groups according to positivity or anergy of tuberculin reaction. Statistical analyses were performed using the Mann-Whitney U test.

Results

The percentage of γ/δ positive cells in peripheral lymphocytes of all the patients with tuberculosis did not differ significantly from the values for healthy individuals; however, tuberculin anergic patients had a significantly higher rate of γ/δ positive cells than tuberculin positive patients ($p<0.001$). The γ/δ cell count in healthy tuberculin positive subjects was low, similar to that in tuberculin positive patients and significantly different from the values in tuberculin negative patients (fig 1).

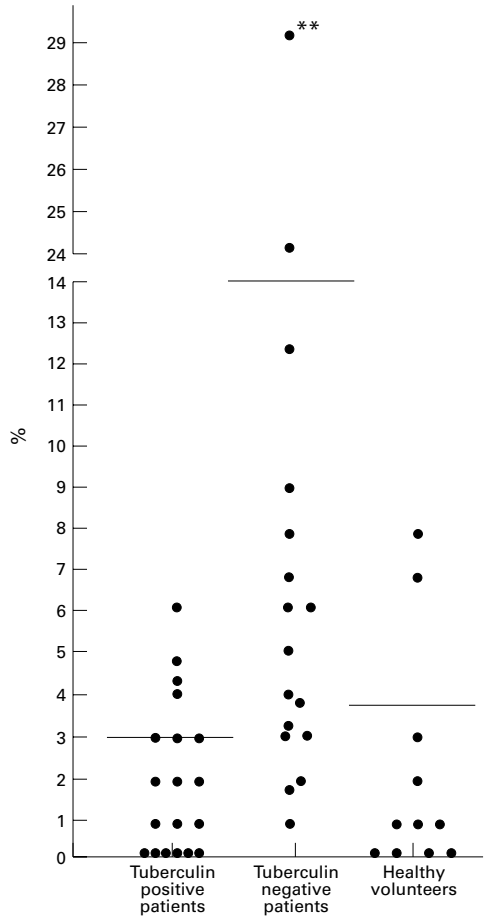

*Figure 1 Percentage of γ/δ positive lymphocytes in peripheral blood of tuberculin positive and tuberculin negative patients and healthy individuals. The horizontal lines represent the mean (SE) of 19, 17, and 11 determinations, respectively. **p<0.001 compared with healthy controls.*

Discussion

Several reports have suggested a protective role for γ/δ T lymphocytes in human *M tuberculosis* infection but the exact role of these cells is still not clear.[7 9] Some authors found no difference in the percentage of peripheral γ/δ T lymphocytes in patients with *M tuberculosis* infection and healthy individuals[6–8] while others observed an increase in the number of γ/δ T lymphocytes in the peripheral blood of patients with tuberculosis.[4 5] The percentage of γ/δ T lymphocytes in our patients with tuberculosis was not significantly higher than that in healthy individuals. However, there was a significantly increased number of γ/δ T lymphocytes in tuberculin negative patients compared with tuberculin positive patients and healthy individuals.

There are no data on the relationship between tuberculin reactivity and γ/δ T cell positivity except for one report in which all patients showed positive tuberculin test reactivity and the percentage of γ/δ T cells did not differ from that of the healthy controls.[6]

The significance of the association between increased numbers of γ/δ T lymphocytes and tuberculin negativity is not clear. These patients did not differ in the course of the disease during chemotherapy or the time needed for recovery from those with positive tuberculin tests and normal γ/δ T lymphocyte numbers.

Isolated γ/δ T lymphocytes from patients with protective immunity to *M tuberculosis* show a higher rate of proliferation when stimulated with the bacterium than those obtained from anergic patients.[7] We found a higher number of γ/δ T cells in tuberculin negative patients than in those positive for *M tuberculosis*; however, the function of these lymphocytes has not been tested. Since γ/δ T cells are known to react with conserved sequences, their role might be one of protection from a potentially harmful autoimmune reaction.

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Bronchoalveolar lavage cell profile in methotrexate induced pneumonitis

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Abstract

***Background* – Pneumonitis is a rare but potentially life threatening side effect of methotrexate treatment for rheumatoid arthritis which needs to be distinguished from interstitial lung disease due to rheumatoid arthritis.**

***Methods* – To examine the value of bronchoalveolar lavage (BAL) in diagnosing methotrexate pneumonitis, the BAL cell profile of four patients with methotrexate pneumonitis was compared with findings in 16 patients with rheumatoid arthritis treated with methotrexate without clinical or radiological evidence of lung disease and eight patients with interstitial lung disease secondary to rheumatoid arthritis treated with methotrexate.**

***Results* – Methotrexate pneumonitis was associated with an increase in the lymphocytes in the BAL fluid to 33–68% of total BAL cells. BAL lymphocytosis was also found in five patients in each of the two control groups. The four patients with methotrexate pneumonitis had a disproportionate increase in CD4+ cells to 72–84% of total lymphocytes and in the CD4/CD8 ratio to 17.0, 6.6, 8.7, and 4.0, respectively, figures which exceeded those of the two control groups.**

***Conclusions* – Methotrexate pneumonitis was associated with lymphocytic alveolitis with a preferential increase in CD4+ cells. This pattern differs from that in interstitial lung disease due to rheumatoid arthritis and may therefore assist in making an early diagnosis of methotrexate pneumonitis.**

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Keywords: rheumatoid arthritis, methotrexate, lung, pneumonitis.

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Pneumonitis is a potentially life threatening side effect of treatment with methotrexate that requires immediate discontinuation of the drug.[1] Characteristically, patients experience a prodromal phase with progressive cough, dyspnoea, and malaise which can last from a few days up to several weeks. At this stage incipient methotrexate pneumonitis needs to be distinguished from interstitial lung disease due to rheumatoid arthritis.[1] This is usually made on clinical grounds such as the presence or absence of constitutional symptoms, the rate of progression, and the response to withdrawal of the drug. While interstitial lung disease due to rheumatoid arthritis is usually a chronic disorder which takes a slowly progressive course and is associated with minor constitutional complaints,[2] methotrexate pneumonitis is an acute and rapidly progressive disorder accompanied by prominent constitutional symptoms.[1]

The value of bronchoalveolar lavage (BAL) in this situation is unclear. We have therefore performed a study of the BAL cell profile and the immunophenotype of BAL lymphocytes in patients with rheumatoid arthritis with methotrexate pneumonitis and compared our findings with those of methotrexate treated patients with rheumatoid arthritis, with and without interstitial lung disease, to see whether characteristics of the BAL fluid help in distinguishing between these disorders.

Methods

Three women and one man aged 59, 66, 60 and 57 years, respectively, with an established diagnosis of seropositive rheumatoid arthritis were diagnosed as having methotrexate-induced pneumonitis. Three of the patients were diagnosed according to the criteria of Carson *et al*,[3] comprising a clinical course consistent with a hypersensitivity reaction, resolving infiltrates on the chest radiograph after discontinuing methotrexate, exclusion of infection or other pulmonary disease, and pathology consistent with drug-induced injury. The presence of any three of these criteria was

Table 1 Pulmonary findings in patients with rheumatoid arthritis with methotrexate induced pneumonitis, patients with rheumatoid arthritis treated with methotrexate without lung disease (methotrexate controls), and methotrexate treated patients with interstitial lung disease due to rheumatoid arthritis (methotrexate + ILD). Values of the control groups are presented as median (95% confidence intervals)

Patient groups	Lung function (% predicted)			P_{O_2} (kPa)	P_{CO_2} (kPa)	BAL cell recovery (10^4 cells/ml)	BAL cell profile (%)			
	VC	FEV_1/VC	TLCO/VA				Lymphocytes	Neutrophils	Eosinophils	Macrophages
Methotrexate pneumonitis										
Patient 1	86	70	58	7.9	4.1	15.1	68	1	1	30
Patient 2	86	91	80	11.6	4.5	21.5	62	3	0	35
Patient 3	107	76	99	9.6	4.4	31.9	33	2	4	61
Patient 4	104	60	76	11.2	4.5	14.3	40	0	0	60
Methotrexate controls (n = 16)										
	101	81	87	11.7	4.6	22.8	10	2	0	89
	(89–108)	(78–84)	(73–97)	(10.0–12.8)	(4.4–4.8)	(9.2–60.6)	(7–18)	(1–3)		(74–92)
Methotrexate + ILD (n = 8)										
	83	81	89	11.1	4.8	23.2	21	3	0	76
	(68–109)	(58–90)	(5–129)	(8.8–15.1)	(4.1–5.1)	(13.4–52.8)	(8–33)	(2–9)		(67–79)

VC = vital capacity; FEV_1/VC = forced expiratory volume in one second/vital capacity; TLCO/VA = carbon monoxide transfer factor corrected for haemoglobin and alveolar volume; P_{O_2}, P_{CO_2} = blood gas tensions; BAL = bronchoalveolar lavage.

required to make a diagnosis of methotrexate pneumonitis. Three patients also had scattered ground glass opacities on high-resolution computed tomographic (HRCT) scanning. Patient no. 3 had a normal chest radiograph and no pathological diagnosis was made but widespread ground glass opacities were seen on the HRCT scan. Current doses of methotrexate were 15 mg/week in patients 1, 2, and 4 and 25 mg/week in patient 3. Further characteristics are presented in table 1.

The first control group comprised 11 women and five men with rheumatoid arthritis who were being treated with methotrexate without clinical, radiological, or functional evidence of interstitial lung disease (methotrexate controls). Their median age was 59 (95% confidence interval 53–61) years, the disease duration was 90 (48–96) months, and the methotrexate dose was 22.5 (15–22.5) mg/week (table 1). The second control group included eight patients (four men) with rheumatoid arthritis who were being treated with methotrexate and who had developed interstitial lung disease secondary to rheumatoid arthritis (methotrexate + ILD). Their median age was 62 (55–69) years, the disease duration was 78 (14–152) months, and the methotrexate dose was 15 (10–25) mg/week. These patients had mild or moderate exertional dyspnoea, which was essentially stable over time, a decreased vital capacity or carbon monoxide transfer factor, and increased interstitial markings on the chest radiograph. None had any appreciable constitutional symptoms suggestive of drug hypersensitivity. At the time of examination the four patients with methotrexate pneumonitis, seven of the eight with interstitial lung disease, and eight of the 16 methotrexate controls were on prednisone, the median daily dose being 7.5 mg in the first two groups and 5 mg in the last.

Bronchoalveolar lavage was carried out in a single lung segment with 240 ml sterile 0.9% saline. The cell differential count was determined by microscopic examination of cytospin preparations stained with May-Giemsa-Grünwald. For immunotyping BAL lymphocytes were labelled with fluorescent anti-CD3, anti-CD4, and anti-CD8 (Coulter, Krefeld, Germany and Becton Dickinson, Heidelberg, Germany) and counted in a Coulter EPICS II flow cytometer. Infection with conventional bacterial pathogens, acid-fast bacteria, *Legionella* species, *Chlamydia* species, *Pneumocystis carinii*, and *Mycoplasma* species was excluded by appropriate methods.

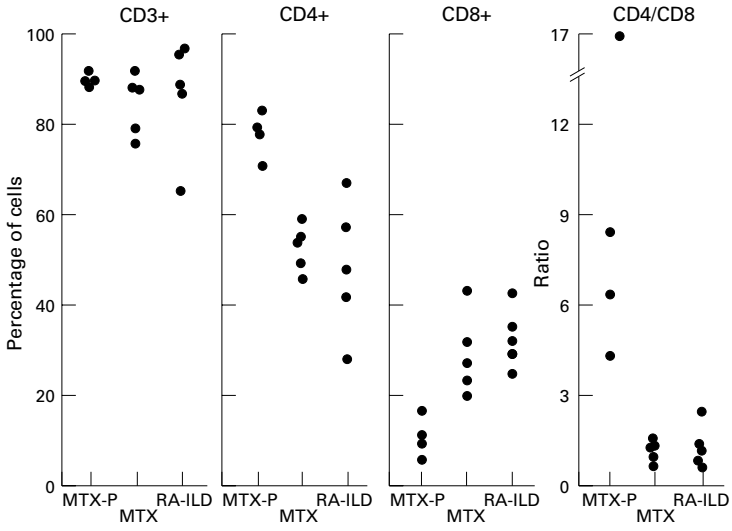

Figure 1 Subsets of lymphocytes in bronchoalveolar fluid of patients with rheumatoid arthritis (RA) with methotrexate pneumonitis (MTX-P), methotrexate treated rheumatoid arthritis without lung disease (MTX), and methotrexate treated rheumatoid arthritis with interstitial lung disease due to rheumatoid arthritis (RA-ILD).

Results

No significant difference was found in the total number of BAL cells recovered from the patients with methotrexate pneumonitis and the two control groups (table 1). All four patients with methotrexate pneumonitis had an increase in the proportion of lymphocytes in the BAL fluid to 68%, 62%, 33%, and 40% (normal in this laboratory <15%). The eosinophil count was normal in all four patients but one patient had an increased proportion of eosinophils to 4% (normal <1%). Five of the 16 methotrexate controls had an increased proportion of lymphocytes with individual values ranging from 18% to 61%, and seven of the eight in the methotrexate + ILD group had an abnormal BAL cell differential with increased lymphocytes (19–52%) in five and increased neutrophils (9% and 12%) in two patients.

Immunotyping of the BAL lymphocytes was performed in the four patients with methotrexate pneumonitis and the five patients in each of the control groups with increased lymphocyte counts. No difference between the groups was found for the percentage of CD3+ cells (fig 1). However, those with methotrexate pneumonitis had an increase in the percentage of CD4+ cells to 84%, 78%, 78%, and 72% of total lymphocytes resulting in CD4/CD8 ratios of 17.0, 6.5, 8.7, and 4.0, respectively. These figures clearly exceeded those of the two control groups.

Discussion

The four patients with methotrexate pneumonitis presented with a non-productive cough and dyspnoea which progressed over a period of 3–8 weeks and were eventually accompanied by profound malaise and myalgia. One patient also developed a fever after the weekly administrations of methotrexate. All had radiographic or HRCT findings suggestive of interstitial lung disease. This picture is compatible with drug hypersensitivity and is well documented for methotrexate induced pneumonitis.[1 3] Withdrawal of methotrexate and a short course of 50 mg prednisone led to a dramatic improvement in all four patients. Tapering of prednisone was tolerated without a relapse in pulmonary or systemic signs or symptoms.

Methotrexate pneumonitis is diagnosed primarily on clinical and radiological grounds. Pulmonary biopsy specimens are of little help since the most characteristic histopathological findings – namely, interstitial infiltration with lymphocytic cells and histiocytes, scattered eosinophils and, occasionally, non-caseating granulomas – have also been observed in interstitial lung disease due to rheumatoid arthritis[4] and do not therefore distinguish between the two disorders.

This study confirms other reports in showing that methotrexate pneumonitis is usually associated with an increase in the proportion of lymphocytes in the BAL fluid.[5–8] However, BAL lymphocytosis is also a fairly common finding in patients with rheumatoid arthritis, with or without clinical and radiological evidence of lung disease, and is an indication of interstitial lung disease due to rheumatoid arthritis.[9 10] To our knowledge, this is the first study to show that methotrexate pneumonitis can be distinguished from interstitial lung disease due to rheumatoid arthritis by a disproportionate increase in CD4+ cells and a raised CD4/CD8 ratio. This is in agreement with two previous reports of methotrexate pneumonitis in patients with cancer[7] and rheumatoid arthritis.[8] In contrast, another study reported a decrease in CD4+ cells in two out of three patients with this condition who received methotrexate as part of a combination regimen.[6] It is therefore questionable whether their lung disease did indeed result from methotrexate intolerance.

Evidence is thus accumulating that methotrexate pneumonitis is generally associated with lymphocytic alveolitis with a disproportionate increase in CD4+ cells. Our study is based on observations in a small number of subjects but suggests that this pattern distinguishes methotrexate pneumonitis from that of low to moderately active interstitial lung disease due to rheumatoid arthritis. In rare cases interstitial lung disease associated with rheumatoid arthritis takes a more aggressive course[2] and, while the BAL cell pattern in such cases has not been determined, this might also be associated with an increase in the CD4+ BAL lymphocytes. To differentiate between lung disease associated with rheumatoid disease and methotrexate pneumonitis physicians should therefore not rely solely on BAL findings but should view these as a useful adjunct to established clinical and radiological criteria for methotrexate pneumonitis.

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