Pulmonary sarcoidosis: alterations in bronchoalveolar lymphocytes and T cell subsets

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ABSTRACT Peripheral blood and bronchoalveolar lavage lymphocyte subpopulations have been evaluated in 14 patients with pulmonary sarcoidosis and eight normal subjects, monoclonal antibodies of the leu series being used. No significant alterations of T lymphocyte subpopulations were found in the peripheral blood of sarcoidosis patients. There was, however, a significantly greater proportion of T suppressor-cytotoxic cells (36.0 (SD 17.6%)) in the bronchoalveolar lavage fluid of patients than of normal subjects (15% (5.6%); $p < 0.01$), but a decrease in the proportion of T helper-inducer cells (51.1% (18%) v 79.3% (9%). These changes correlated with the duration of the disease but not with other clinical, radiological, physiological, or biochemical criteria. Patients were followed up for six to 20 months and five patients had a repeat bronchoalveolar lavage and lymphocyte subpopulation evaluation after three to 14 months. The initial pulmonary T lymphocyte subset proportions were not predictive of clinical, physiological, or radiological alterations during follow up. There was also no consistent pattern in the relationship between change in T subset proportions and change in clinical physiological, and radiological features in the five patients having a repeat lavage. Lymphocyte surface marker studies may indicate immunopathogenetic mechanisms in sarcoidosis but do not appear to be good predictors of clinical outcome.

Various alterations in immune responses have been reported in sarcoidosis.¹⁻³ Cutaneous anergy to tuberculo-protein and other recall antigens is common.^{4,5} Reduced numbers of circulating T lymphocytes are frequent.^{6,7} Peripheral blood lymphocyte functions, as judged by spontaneous transformation or proliferative responses to mitogens *in vitro*, are often abnormal,^{8,9} though not invariably.¹⁰ Hunnighake *et al.*,¹¹ however, have shown that the findings in peripheral blood may fail to reflect the events at sites of active disease. It is now well established that pulmonary sarcoidosis is characterised by a lymphocytic alveolitis, due to an increased number of T lymphocytes.¹² These T cells are activated, spontaneously secreting monocyte chemotactic factor,¹³ helper factor for B lymphocyte differentiation,¹⁴ and interleukin 2,¹⁵ thereby contributing to the immunopathological processes of pulmonary sarcoidosis.

It has been suggested that knowing the proportion of T lymphocytes in cells obtained at bronchoalveolar lavage may help in the evaluation of pulmonary sarcoid.¹⁶ Patients at most risk of deteriorating pulmonary function at six month follow up displayed an increase in the helper-inducer subsets in one study.¹⁷ We have therefore examined the pulmonary and peripheral blood T lymphocyte numbers and subtypes of patients with pulmonary sarcoidosis of varying activity and duration to compare the pattern of cells in bronchoalveolar lavage fluid and in peripheral blood, and to determine its relevance in assessing clinical state and predicting outcome of the disease.

Methods

PATIENTS

Fourteen patients with sarcoidosis (proved by biopsy) of varying duration and activity were studied. Clinical and investigative details are shown in table 1. No patient had received corticosteroid treatment during the year before study. Controls

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Table 1 Individual clinical, radiological, and physiological, details of 14 patients with pulmonary sarcoidosis, related to bronchoalveolar lymphocytes and T cell subsets obtained at time of initial bronchoalveolar lavage (all patients were non-smokers apart from 1, 5, and 11, who were ex-smokers)

Patient No	Sex	Age (y)	Disease duration (months)	Chest radiograph ^{1a} (stage)	Pulmonary function tests			Bronchoalveolar lavage lymphocytes			Alterations at follow up [†]			
					FEV ₁ /VC (%)	KCO (% predicted)	TLC	% total cells	leu 3a+ % T cells (mean (SD))	leu 2+	Chest radiograph	FEV ₁ /VC	KCO	TLC
1	F	29	2	I	80	ND**	ND	24	52 (9)	25 (5)	+	0	ND	ND
2	M	28	2	I	81	78	100	48	63 (8)	27 (7)	+	0	0	0
3	F	42	5	III	74	106	70	54	84 (6)	9 (7)	+	+	0	+
4	F	47	8	I	80	97	84	43	39 (4)	37 (5)	+	0	0	0
5	M	57	12	II	54	93	68	53	68 (4)	32 (7)	NFU	NFU	NFU	NFU
6	M	61	18	I	58	115	93	55	60 (20)	22 (7)	0	+	0	0
7	M	29	18	III	72	67	73	79	78 (8)	14 (4)	+ ^s	0	+	+
8	F	35	19	II	81	94	74	77	74 (4)	19 (8)	0	0	0	0
9	M	38	20	III	82	65	93	39	60 (15)	18 (8)	NFU	NFU	NFU	NFU
10	F	33	22	III	80	114	88	20	35 (14)	65 (7)	0	0	0	0
11	M	29	23	II	77	100	86	14	22 (6)	65 (14)	—	—	—	—
12	F	45	36	III	77	118	90	40	39 (4)	58 (7)	0	0	0	0
13	F	58	60	III	78	107	88	15	39 (18)	47 (12)	0 ^s	0	0	0
14	F	44	96	III	85	94	110	16	23 (13)	48 (23)	0	0	0	0

*Total T cells identified by monoclonal antibody leu 1a, helper-inducer cells by leu 3a, suppressor-cytotoxic cells by leu 2a.

†0 indicates no change in chest radiograph, <10% alteration in results of pulmonary function tests; + indicates reduction in hilar adenopathy or interstitial shadows or both on radiograph, >10% improvement in results of pulmonary function tests; — indicates increase in interstitial shadows on radiograph, >10% deterioration in results of pulmonary function tests; NFU—no follow up; s—steroid treatment.

**Not done.

VC—vital capacity; KCO—transfer coefficient; TLC—total lung capacity.

were eight healthy, normal volunteers (four of them men) of mean age 33 years (range 27–51 years). Two were ex-smokers and the remainder non-smokers. The subjects gave informed consent and the local ethics committee approved the study. Cells for study were acquired by venesection and bronchoalveolar lavage.

Twelve patients were followed in the clinic for six to 20 (mean 12.3) months. The changes in the chest radiographic findings and results of pulmonary function tests at the latest follow up are shown in table 1.

Two patients who moved away from the area were not available for follow up. Five patients had a second bronchoscopy and bronchoalveolar lavage three to 14 months after the initial bronchoalveolar lavage (table 2).

BRONCHOALVEOLAR LAVAGE

Bronchoalveolar lavage¹⁹ was performed by wedging a fiberoptic bronchoscope (Olympus BF 1 T) in a subsegmental bronchus of the middle lobe or lingular. A total of 300 ml of sterile physiological saline

Table 2 Bronchoalveolar lymphocytes, T cell subsets, and alterations in disease activity for five patients at follow up bronchoalveolar lavage

Patient No	Lavage No	Disease duration (months)	BAL cells (% lymphocytes)	BAL cells leu 3a+ (% total T, mean (SD))	BAL cells leu 2a+ (% total T, mean (SD))	Change in disease activity and treatment between first and second lavage
3	1	5	54	84 (6)	9 (7)	No steroids; radiograph unaltered; no change in results of pulmonary function tests but subsequently improvement in both radiograph and pulmonary function
	2	13	44	64 (8)	39 (11)	
7	1	18	79	78 (8)	14 (4)	Steroids given; reduction of interstitial shadowing on radiograph; improvement in results of pulmonary function tests; ACE and gallium scan became normal
	2	21	68	76 (9)	19 (10)	
8	1	19	77	74 (4)	19 (8)	No steroids; no change in radiograph or results of pulmonary function tests but sarcoid hepatic and skin lesions developed
	2	33	69	88 (12)	17 (14)	
11	1	23	14	22 (6)	65 (14)	No steroids; developed symptoms; slight worsening of interstitial shadowing on radiographs; decrease in TLC and KCO; ACE also rose
	2	32	26	67 (22)	40 (6)	
13	1	60	15	39 (18)	47 (12)	Steroids given; no change in radiograph or results of pulmonary function tests
	2	70	14	28 (5)	69 (13)	

*Total T cells identified by monoclonal antibody leu 1a, helper-inducer cells by leu 3a, and suppressor-cytotoxic cells by leu 2a.

ACE—angiotension converting enzyme; TLC—total lung capacity; KCO—transfer coefficient.

Fig 1 Lymphocytes expressed as percentages of total bronchoalveolar lavage (BAL) cells: data from patients with sarcoidosis plotted against the chest radiographic stage¹⁸ and duration of disease.

was instilled in 30 ml aliquots with immediate aspiration into an ice cold trap. An equal volume of medium 199 (Gibco Ltd, Paisley) with 2.5% HEPES buffer pH 7.3 (Gibco) was immediately added to the lavage fluid.

Bronchoalveolar cells were recovered by low speed centrifugation ($100 g \times 10 \text{ min}$) at 4°C , suspended in medium 199, and counted. Differential cell counts of aliquots of bronchoalveolar lavage cells were made on cytocentrifuge preparations (Cytospin, Shandon), a modified Giemsa stain (Diff-Quik, American Hospital Supply Co, Didcot) being used and 500 cells counted.

T LYMPHOCYTE SUBPOPULATIONS

Mononuclear cells were separated from peripheral blood and from the resuspended bronchoalveolar lavage cells by density centrifugation over Ficoll-Hypaque. Adherent and non-adherent cells from the mononuclear fraction were separated by plastic adherence. The cells at a density of $1 \times 10^6/\text{ml}$ were allowed to adhere to a 15 cm tissue culture dish (Sterilin, Teddington) for one hour at 37°C . Non-adherent cells were removed from the dishes by three washes with Hanks balanced salt solution

(HBSS) and resuspended in culture medium RPMI 1640 (Gibco) at 3×10^6 cells/ml. Contamination of this non-adherent population by monocytes or macrophages was under 2% as judged by morphological examination and esterase staining. The non-adherent population was examined for total T cells and proportions of T cell subpopulations by direct immunofluorescence, monoclonal antibodies of the leu series (Becton Dickinson, Twickenham, England) being used. Fluorescein labelled antibodies leu 1a, leu 2a, and leu 3a were used to identify respectively the total T cells, the T suppressor-cytotoxic subpopulations, and the T helper-inducer subpopulations.²⁰ Briefly, 1.5×10^6 cells were incubated with 1:200 dilutions of monoclonal antibodies on ice for one hour. The cells were then washed extensively, resuspended in phosphate buffered Minimal Essential Medium pH 7.4 containing 0.1% (w/v) sodium azide, 2% (v/v) newborn calf serum, and 50% (v/v) glycerol (v/v), and examined by fluorescence microscopy. T cell subpopulations are expressed as mean percentages of the total T cells, as identified by the leu 1a antibody, with one standard deviation in parentheses.

Statistical analyses were by analysis of variance.

Results

Patients with sarcoidosis had a greater total number of cells in the bronchoalveolar lavage fluid (2.94 ($SD 1.39$) $\times 10^7$) than normal subjects (1.90 (1.32) $\times 10^7$) and a greater proportion were lymphocytes (42.2% (22%) than in normal subjects (7.7% (5.8%)) (fig 1). Thus sarcoidosis patients had 4.52 (1.32) $\times 10^6$ lymphocytes compared to 1.87 (1.24) $\times 10^6$ in normal subjects.

The proportions of T helper-inducer and T suppressor-cytotoxic cells in the peripheral blood of patients with sarcoidosis did not differ significantly from those of normal subjects (table 3, fig 2). In the lung, the proportion of T suppressor-cytotoxic cells was significantly greater and of T helper-inducer cells significantly smaller than in normal subjects ($p < 0.01$) (table 3). Within the patient group the variations in the T cell subpopulations were consider-

Table 3 T lymphocyte subpopulations in the blood and lungs of normal subjects and patients with pulmonary sarcoidosis

	Peripheral blood		Bronchoalveolar lavage fluid	
	leu 3a ⁺ Mean (SD)	leu 2a ⁺ % of T lymphocytes*	leu 3a ⁺ Mean (SD)	leu 2a ⁺ % of T lymphocytes
Normal subjects (n = 8)	73.0 (9.9)	17.8 (6.4)	79.3 (8.7)	15.0 (5.6)
Sarcoidosis patients (n = 14)	69.1 (8.0)	21.9 (5.7)	51.1 (18.3)†	36.0 (17.6)†

*Total T cells identified by monoclonal antibody leu 1a, helper-inducer cells by leu 3a, and suppressor-cytotoxic cells by leu 2a.

† $p < 0.01$ for comparison of patients with sarcoidosis with normal subjects.

Fig 2 T lymphocyte subsets leu 3a⁺ (●, helper-inducer cells) and leu 2a⁺ (○, suppressor-cytotoxic cells) in peripheral blood and bronchoalveolar lavage cells from eight normal subjects and 14 patients with pulmonary sarcoidosis. Each is expressed as a percentage of total T cells (as assessed by monoclonal antibody leu 1a). in panel (a) the data are plotted against disease duration and in panel (b) against chest radiographic stage.¹⁸

able (fig 2). Patients with recent onset or intermediate duration of disease (0–20 months) had in general moderate or large increases in percentages of lymphocytes in their bronchoalveolar lavage fluid (fig 1) but relatively normal proportions of leu 3a⁺ and 2a⁺ cells (fig 2a), suggesting that their alveolitis was due to increases in both helper-inducer and suppressor-cytotoxic cells. Patients with longer disease duration (over 20 months) had almost normal percentages of bronchoalveolar lavage lymphocytes (fig 1) but a significantly increased proportion of suppressor-cytotoxic cells and a decreased proportion of helper-inducer cells ($p < 0.01$; fig 2a), indicating a less florid cellular alveolitis with appreciable imbalance of T cell subsets. This small group included some patients whose disease was considered on clinical grounds to be inactive or resolving and some

who had clinically chronic active disease. There was no statistically significant association between other characteristics of patients (table 1), including the chest radiographic stage (fig 2b) and the increased proportion of suppressor-cytotoxic cells.

Neither the percentage of lymphocytes in the bronchoalveolar lavage fluid nor the T lymphocyte subpopulations appeared to show associations with the clinical, radiological, or physiological progress of the disease (table 1). This observation is supported by the data on repeat lavage in five patients (table 2), though the small size of this group, together with the fact that some received steroids while others did not, prevents firm conclusions from being drawn from the data. It should be noted that the one patient (No 7) who showed dramatic clinical, radiological, and physiological improvement had minimal changes in the percentage of bronchoalveolar lavage lymphocytes and no change in the proportion of leu 3a⁺ and leu 2a⁺.

Discussion

Our findings of an increased percentage of lymphocytes in the bronchoalveolar lavage cells from patients with sarcoidosis are consistent with previously described data.^{11 21 22} The percentage of lymphocytes in the bronchoalveolar lavage did not correlate with any of the standard measures we made (table 1), although patients with disease of over 20 months' duration tended not to have increased lymphocytes. This observation should be interpreted with caution since numbers of patients were small and "duration" of disease is imprecise. We took "duration" to be the time between objective diagnosis or onset of symptoms that could reasonably be attributed to sarcoidosis (for example, arthralgia, erythema nodosum) and bronchoalveolar lavage. Clearly, for some patients this will result in an underestimate of disease duration.

In sarcoidosis, where disease activity may vary between different organs, the lymphocyte subpopulations in peripheral blood may differ significantly from those in, for example, the lung.¹¹ Our data (fig 2) would indicate that this is also true for T cell subpopulations. In accord with Ginns *et al*²³ we found that the proportions of these subpopulations in peripheral blood were not significantly different from those of normal subjects. Hunninghake and Crystal,¹⁷ however, have reported a small increase in T suppressor-cytotoxic cells in peripheral blood. There were methodological differences since we used monoclonal antibodies of the leu series whereas they used the OK T series, our total T cell population was identified by monoclonal antibody leu 1a and theirs by sheep red cell rosetting, and our

T cell subpopulations were determined on cells purified by adherence and theirs by rosetting. There may also have been differences in selection of patients since our patients were all primary referrals.

In the bronchoalveolar lavage cells we found different proportions of T cell subpopulations in patients with sarcoidosis and in normal subjects (table 3, fig 2). There was appreciable interpatient variability, with large changes even when the proportions of peripheral blood subpopulations were normal. Our grouped data with increased proportions of T suppressor-cytotoxic cells contrast with the findings of other published series,^{17 23 24} where the proportion T helper-inducer cells was increased. Some of these differences may result from different methods or selection of patients (see above), but could also arise by the grouping of data. Our mean results are affected by the subgroup of patients with longer disease duration. Hunninghake and Crystal¹⁷ divided their patients before analysis and found one subgroup with an appreciable increase of helper-inducer T cells and another with a smaller increase of suppressor-cytotoxic T cells; their combined data had a scatter of results similar to ours. Ginns *et al*²³ studied only six patients and had no data from normal subjects for comparison. Ceuppens and coworkers²⁴ recently reported data on a larger group, 35 patients. Although the mean percentage of helper-inducer cells was greater in their patients and of suppressor-cytotoxic cells smaller than in ours, there is considerable overlap. Their patient population probably differed from ours, since only nine out of 31 (29%) untreated patients had stage III chest radiographs compared with seven out of 14 (50%) in our series.

The only clinical criterion that was associated with increased proportions of T suppressor-cytotoxic cells was disease duration. Four of the five patients with disease duration of over 20 months had a stage 3 chest radiograph; but too many other patients had stage 3 changes to make it a useful discriminator (fig 2b). Recent data²⁵ have indicated the importance of not overinterpreting changes in helper-inducer and suppressor-cytotoxic proportions and we would not wish to overemphasise the relationship of disease duration to T cell subsets, preferring to speculate that both "disease duration" and bronchoalveolar lavage T cell subset imbalance vary with pulmonary immunopathogenetic mechanisms. Rossi and colleagues found an expansion of a subpopulation of helper T cells in a group of patients with sarcoidosis with no overall increase in helper-inducer cells (OK T 4) but a small relative increase of suppressor-cytotoxic cells (OK T 8).

Over the period of follow up only one patient showed deterioration of pulmonary disease and so it

was not possible to determine whether the percentage of lymphocytes, or proportions of T cell subsets, in bronchoalveolar lavage fluid could act as predictors of poor prognosis. These markers were, however, unable to differentiate between patients who showed objective improvement and those who did not (table 1). The limited number of patients available for repeat lavage prevented firm conclusions being drawn from the data. The decrease in percentage of helper-inducer cells from patient 3 (table 2) and her subsequent improvement and the increase in percentage of helper-inducer cells from patient 11 (table 2) in association with his deterioration are consistent with the proposal of Hunninghake and Crystal¹⁷ that active disease is associated with helper T lymphocyte activity. The considerable clinical, physiological and radiological improvement of patient No 7 (table 2), however, occurred in the face of minimal reduction in the percentage of bronchoalveolar lavage lymphocytes and no change in leu 3a⁺ or leu 2a⁺ T cell subsets. This raises the possibility that bronchoalveolar lavage lymphocyte numbers and subtypes are simply reflecting the current immunopathological process, rather than containing intrinsic prognostic information. This observation, in conjunction with the findings of Rossi *et al*,²⁵ suggests that study of the function of lymphocytes sampled by bronchoalveolar lavage might be more informative in predicting clinical progress than enumeration of lymphocytes or lymphocyte subpopulations.

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