

# T and B lymphocytes in sarcoidosis: a clinical correlation

J. M. CUMMISKEY<sup>1</sup>, H. McLAUGHLIN,  
and P. KEELAN

*Mater Misericordiae Hospital, Dublin 7 and Department of Pathology,  
University College, Dublin 2*

**Cummiskey, J. M., McLaughlin, H., and Keelan, P. (1976). *Thorax*, 31, 665–668. T and B lymphocytes in sarcoidosis: a clinical correlation.** Immunological abnormalities in sarcoidosis have been previously described. Cutaneous anergy to a wide variety of antigens first prompted the suggestion that the underlying defect may be of importance in the aetiology or pathogenesis of the disorder. The thymus derived lymphocytes appear to be particularly affected, and both quantitative and qualitative *in vitro* defects have been described in these cells in sarcoidosis patients. We have quantitatively investigated T and B cells in a series of 52 sarcoidosis patients, and our results indicate that, as a group, sarcoidosis patients have lower mean total lymphocyte counts and lower T cell counts than the control series in agreement with other reports. We found no difference between B cells in the sarcoidosis and control groups. The quantitative abnormalities detected did not correlate with any of the clinical parameters which were investigated—stage of disease, duration of the disease, treatment regime, and activity of disease—and there was a considerable overlap between the results obtained in sarcoidosis patients and the controls. Our results indicate that these investigations are of little value in the management of sarcoidosis patients.

*In vitro* investigations of immunological reactivity in sarcoidosis have revealed both quantitative and qualitative abnormalities in the immune system. Lymphopenia has been reported as a feature in a high percentage of patients (Hoffbrand, 1968), and the mean total lymphocyte count of groups of sarcoidosis patients has been shown to be significantly lower than that of control groups (Hedfors, Holm, and Pettersson, 1974; Ramachandar *et al.*, 1975). Studies on lymphocyte subpopulations have shown that the lymphopenia is due to a selective reduction in thymus derived lymphocytes (T cells) responsible for cellular immune responses while 'Bursa' derived lymphocytes (B cells) responsible for humoral immunity have been found to be present in normal or increased numbers (Hedfors *et al.*, 1974; Mangi *et al.*, 1974a; Ramachandar *et al.*, 1975). Techniques available for identifying these subpopulations include the sheep red cell rosette technique for T cells (Jondal, Holm, and Wigzell,

1972; Wybran, Carr, and Fundenberg, 1972) and a fluorescent antibody technique for the identification of immunoglobulin bearing cells (B cells) (Papamichail, Brown, and Holborow, 1971; Brown and Greaves, 1974).

We report here a quantitative analysis of T and B cell populations in a group of 52 patients with sarcoidosis. Findings have been correlated with the clinical features of these patients to determine the value of these investigations in a clinical context.

## MATERIAL AND METHODS

**PATIENTS AND CONTROLS** The series consisted of 52 patients (mean age 31 years) with sarcoidosis and 34 healthy young adults (mean age 25 years) as controls. The diagnosis of sarcoidosis was confirmed histologically in all patients. All have been examined clinically, radiologically, and with pulmonary function tests within 12 months of this study.

The patients were grouped according to the following clinical criteria: stage, activity, dura-

<sup>1</sup>Present address: Division of Pulmonary Medicine, Department of Medicine, Stanford University Medical Center, Stanford, California

tion of disease process, and treatment programme (Table I). The stage of the disease depended on the chest radiograph: stage I had bilateral hilar lymphadenopathy (BHL), stage II had BHL and pulmonary infiltrates, and stage III had pulmonary infiltrates with or without fibrosis. Patients with a clear chest radiograph were classified as extrathoracic sarcoidosis (stage 0). Disease was considered to be active if its onset was within the preceding 12 months, if new clinical symptoms or signs had appeared, if pulmonary infiltrates showed either no change or progression of lesions, or if pulmonary function tests were unchanged or had deteriorated within the preceding year. A patient was included in the 'treated' group if he or she was currently receiving prednisone or had received prednisone for more than three weeks within the preceding six months.

TABLE I

## COMPARISON OF SUBGROUPS WITHIN SARCOID GROUP

1. Stage I disease	(26)	∨	Stages II, III, and 0	(26)
2. 'Active' disease	(42)	∨	'Inactive' disease	(10)
3. Patients on treatment	(27)	∨	Patients off treatment	(25)
4. Duration < 2 years	(33)	∨	Duration > 2 years	(19)

The number of patients in each subgroup is shown in parentheses.

## INVESTIGATIONS

**Total lymphocyte count** This was derived from the total white cell count (obtained in Coulter F machine) and a differential white cell count.

**Lymphocyte separation** Fresh heparinized blood was layered on a Ficoll Hypaque density gradient and spun in a centrifuge for 20 min at 1500 rpm. Cells aspirated from the interface were washed in Hanks' solution three times. A drop was diluted in 2% glacial acetic acid for counting and cells were resuspended at a final concentration of  $2.5 \times 10^6$ /ml in Hanks' solution.

**Labelling of Ig bearing cells** Lymphocytes prepared as outlined above were centrifuged. The button was resuspended in 5 drops of 1/5 solution fluorescein conjugated polyvalent antiserum to human immunoglobulin G, A, and M (Wellcome). Cells were incubated for 30 minutes at 4°C, washed three times, and examined with a Zeiss standard Universal microscope fitted with a mercury superpressure lamp as ultraviolet (UV) source. Primary filters BG12 and BG38 and a secondary filter with a cut-off at 5300Å were used. Each field was examined under UV light and

ordinary light alternately to obtain the percentage labelled cells.

**Sheep red blood cell rosette test** Sheep red blood cells (SRBC) were washed three times in Hanks' solution and adjusted to a final concentration of 0.5% ( $1 \times 10^8$ /ml). To 0.2 ml aliquots of lymphocytes (containing  $2.5 \times 10^6$ /ml) were added 0.2 ml SRBC suspension and 10% fetal calf serum. Cells were mixed, centrifuged at 50 g for 10 minutes, and incubated at 4°C overnight. Immediately before counting cells were gently resuspended by shaking. All lymphocytes binding three or more SRBC were considered positive. Two hundred lymphocytes were counted and a percentage was obtained (Jondal *et al.*, 1972).

**Null cells** The absolute number of cells in each preparation from each patient which failed to react with SRBC or anti-immunoglobulin antisera was derived by subtracting the combined totals of T and B lymphocytes from the total lymphocyte count.

**Statistics** Statistical evaluation of the results was carried out with the use of Student's *t* test. Mean values are expressed  $\pm$  standard error of the mean.

## RESULTS

The results of the sarcoidosis series and controls are shown in Table II.

**Total lymphocyte count** The mean total lymphocyte count in the sarcoidosis patients was  $1606/\text{mm}^3 \pm 90$  (SE). This was significantly lower than the mean value obtained in the control series of  $2234/\text{mm}^3 \pm 123$  (SE) ( $P < 0.001$ ).

**T cell count** The mean and standard error obtained in the sarcoidosis group for T cell count was  $1062/\text{mm}^3 \pm 71$ . This again was significantly lower than that seen in the control group,  $1650/\text{mm}^3 \pm 92$  ( $P < 0.001$ ).

**B cell count** The mean and standard error for B cell counts in both groups was  $263/\text{mm}^3 \pm 19$  (sarcoidosis patients) and  $311/\text{mm}^3 \pm 26$  (controls). The difference was not statistically significant.

**Percentage values for all cell counts** The mean percentage values obtained for B and T cell counts are shown in Table II. The mean total percentage of cells identified as either B or T cells in the sarcoid group was 81.7% and in the control group 88.4%. Although the percentage of cells not identified as either B or T cells (null cells) was higher in the sarcoid group than in the controls, the mean and standard error of the absolute

TABLE II

RESULTS OBTAINED FOR TOTAL LYMPHOCYTE COUNT AND T AND B CELL COUNTS IN SARCOIDOSIS PATIENTS AND CONTROLS EXPRESSED AS MEAN AND SE OF MEAN AND AS MEAN % VALUE FOR T AND B CELLS

	Total Lymphocyte Count (mean ± SE)	T Cells		B Cells	
		Number (mean ± SE)	Mean %	Number (mean ± SE)	Mean %
Sarcoidosis patients (52)	1606 ± 90	1062 ± 71	65.4	263 ± 19	16.3
Controls (34)	2234 ± 123	1650 ± 92	74.4	311 ± 26	14.0
Significance	P < 0.001	P < 0.001		N.S.	

TABLE III

RESULTS OBTAINED IN DIFFERENT GROUPS (EXPRESSED AS MEAN ± SE)<sup>1</sup>

	Total Lymphocyte Count	T Cell Count	B Cell Count
Stage: I	1650 ± 146	1126 ± 114	249 ± 28
II, III and 0	1563 ± 107	982 ± 83	276 ± 27
Activity: Active	1571 ± 99	1004 ± 76	266 ± 21
Inactive	1754 ± 216	1284 ± 166	252 ± 49
Duration: < 2 yr	1571 ± 107	996 ± 80	266 ± 22
> 2 yr	1668 ± 163	1166 ± 137	258 ± 36
Treatment: On	1531 ± 105	976 ± 80	278 ± 27
Off	1688 ± 148	1139 ± 118	248 ± 27

<sup>1</sup>Differences between results within subgroups not statistically significant.

numbers of these cells showed no significant difference between the two groups (sarcoidosis patients  $293/\text{mm}^3 \pm 29$  and controls  $272/\text{mm}^3 \pm 40$ ).

**Subgroup analysis** No significant difference was observed in the results obtained within any of the clinical subgroups of sarcoidosis patients analysed for total lymphocyte count, T cell or B cell counts. The results obtained for each clinical subgroup expressed as mean and standard error are shown in Table III.

## DISCUSSION

The results of this study show that sarcoidosis patients as a group have a lower mean total lymphocyte count than normal controls. They also have a significantly lower T cell count while there was no significant difference in the absolute numbers of B cells or null cells between the two groups. Analysis of lymphocyte subpopulations in the clinical subgroups shows that there is no significant difference in the groups selected for stage, activity, treatment programme or duration of the disease.

These results confirm those of several other workers (Hoffbrand, 1968; Hedfors *et al.*, 1974; Ramachandar *et al.*, 1975). There appears to be general agreement on the reduced total lymphocyte count and T cell count in sarcoidosis patients but an elevated mean B cell count has been pre-

viously reported (Ramachandar *et al.*, 1975) although we did not find this in the present study in agreement with other reports (Hedfors *et al.*, 1974; Mangi *et al.*, 1974a). Absolute numbers of cells which fail to react with SRBC and anti-immunoglobulin antisera (null cells) showed no significant difference between the sarcoidosis group and the controls in this study. The nature of these cells is not certain. Undoubtedly some represent contaminating monocytes. The combined percentage total for B and T cells in the present survey was 81.7% for the sarcoidosis group and 88.4% for the control group. Other reports show varying figures for the combined total of T and B cells from 71% to 108% (Mangi *et al.*, 1974a; Ramachandar *et al.*, 1975). This underlines the importance of technical factors in different surveys which make direct comparison difficult. Other problems encountered on comparing different studies on sarcoidosis patients include the variability of the disease process under investigation both within a country and between countries (Hall *et al.*, 1969; Siltzbach *et al.*, 1974).

The failure to demonstrate differences between sarcoidosis patients selected for stage, activity, treatment regime, and duration of the disease process is at variance with some previous reports. A lower total lymphocyte count was found in patients with long-standing disease than in those

of recent onset (Hoffbrand, 1968; Hedfors *et al.*, 1974). A lower T cell count was found in patients receiving corticosteroid therapy than in those not receiving such therapy (Mangi *et al.*, 1974a). However, other studies have failed to show a correlation between quantitative abnormalities of the lymphocyte subpopulations and disease activity (Hedfors, 1975; Ramachandar *et al.*, 1975).

The significance of the observed abnormalities remains to be elucidated. The reduced T cell count in sarcoidosis patients may reflect decreased production or increased 'utilization'. Circulating inhibitors have been identified in sarcoidosis patients which reduced lymphocyte responsiveness to mitogens (Belcher, Carney, and Nankervis, 1974; Mangi, Dwyer, and Kantor, 1974b). A similar mechanism may be involved in the quantitative reduction in T cells. It has been suggested that lymph node involvement by the disease process may impair recirculation of T lymphocytes contributing to the lower T cell counts (Ramachandar *et al.*, 1975).

The results of the present study and others indicate that these investigations are of limited value in the management of individual patients with sarcoidosis. The observed abnormalities in this study fail to correlate with the clinical features of sarcoidosis in groups of patients. The value of sequential analysis in individual patients remains to be assessed.

We wish to thank the Medical Research Council of Ireland for financial support and Miss J. Woods for statistical advice, and Mrs. M. Melinn and Miss O. Crowley for technical assistance.

#### REFERENCES

- Belcher, R. W., Carney, J. F., and Nankervis, G. A. (1974). Effect of sera from patients with sarcoidosis on *in vitro* lymphocyte response. *International Archives of Allergy and Applied Immunology*, **46**, 183.
- Brown, G. and Greaves, M. F. (1974). Enumeration of absolute numbers of T and B lymphocytes in human blood. *Scandinavian Journal of Immunology*, **3**, 161.
- Hall, G., Naish, P., Sharma, O. P., Doe, W., and James D. G. (1969). The epidemiology of sarcoidosis. *Postgraduate Medical Journal*, **45**, 241.
- Hedfors, E. (1975). Influence of steroid treatment on lymphocyte response in sarcoidosis. *Clinical Immunology and Immunopathology*, **4**, 96.
- Hedfors, E., Holm, G., and Pettersson, D. (1974). Lymphocyte subpopulations in sarcoidosis. *Clinical and Experimental Immunology*, **17**, 219.
- Hoffbrand, B. I. (1968). Occurrence and significance of lymphopenia in sarcoidosis. *American Review of Respiratory Diseases*, **98**, 107.
- Jondal, M., Holm, G., and Wigzell, H. (1972). Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming non-immune rosettes with sheep red blood cells. *Journal of Experimental Medicine*, **136**, 207.
- Mangi, R. J., Dwyer, J. M., Gee, B., and Kantor, F. S. (1974a). The immunological competence of subjects with sarcoidosis. *Clinical and Experimental Immunology*, **18**, 505.
- Mangi, R. J., Dwyer, J. M., and Kantor, F. S. (1974b). The effect of plasma upon lymphocyte response *in vitro*. Demonstration of a humoral inhibitor in patients with sarcoidosis. *Clinical and Experimental Immunology*, **18**, 519.
- Papamichail, M., Brown, J. C. and Holborow, E. J. (1971). Immunoglobulins on the surface of human lymphocytes. *Lancet*, **2**, 850.
- Ramachandar, K., Douglas, S. D., Siltzbach, L. E., and Taub, R. N. (1975). Peripheral blood lymphocyte subpopulations in sarcoidosis. *Cellular Immunology*, **16**, 422.
- Siltzbach, L. E., James, D. G., Neville, E., Turiaf, J., Battesti, J. P., Sharma, O. P., Hosoda, Y., Mikami, R., and Odaka, M. (1974). Course and prognosis of sarcoidosis around the world. *American Journal of Medicine*, **57**, 847.
- Wybran, J., Carr, M. C., and Fundenberg, H. H. (1972). The human rosette forming cell as a marker for a population of thymus derived cells. *J. clin. Invest.*, **51**, 2527.

Requests for reprints to: Dr. H. McLaughlin, Department of Pathology, University College, Dublin 2.