Lymphocyte Subsets in Tissue, Blood, and Bronchoalveolar Lavage Fluid in Sarcoidosis *

Om P. Sharma, M.D.

SUMMARY Immuno-peroxidase technique using monoclonal antibodies against the surface antigen of T lymphocytes was employed in six patients with histologically proven cases of active sarcoidosis. The sequestration of helper T cells occurred in granulomatous areas, whereas the patients' peripheral blood had low helper-cell counts. This pattern is similar to one observed in cases of tuberculoid leprosy and suggests that the localisation of T-cell subpopulations might in some way be related to the presence of an as yet undetected antigen which causes the granulomatous reaction.

ASIAN PACIFIC J ALLERG IMMUN 1985; 3:60-62.

Sarcoidosis is a granulomatous disorder of unknown cause. The disease is characterised by hyperactivity of cellular as well as circulating immune systems. The cell-mediated immunity changes are important in the formation of granulomas which are the focal points of lymphocytic responses to yet unknown antigens in sarcoidosis.

A number of attempts have been made to study the lymphocytes that form an integral part of the granulomatous inflammation. In the present study, immuno-peroxidase technique using monoclonal antibodies against surface antigen of T-lymphocytes was employed to study the helper/suppressor T-cell population in sarcoidosis tissue. The helper/suppresser ratio in the peripheral blood and in the bronchoalveolar lavage fluid was studied simultaneously.

MATERIALS AND METHODS

The diagnosis of sarcoidosis was established by clinical or radiological features and histological evidence of non-caseating granulomata. Bacterial and fungal causes were excluded by appropriate laboratory and immunological tests.

Bronchoalveolar lavage

Fibreoptic bronchoscopy was performed after anaesthetising the pharynx and airways with 2% lidocaine spray. The tip of the bronchoscope was wedged in a subsegmental airway of the lingula, where 100 ml of sterile saline were injected in aliquots and aspirated immediately. The usual yield was 40-60 ml of lavage fluid. Lavage fluid cells, obtained by centrifugation, were analysed for total and differential counts.[2]

On the same occasion, at least six transbronchial lung biopsy specimens were obtained from different sites.

Peripheral blood lymphocyte subsets

The percentages of T-helper and T-suppressor lymphocytes and their ratio were determined by the fluorescence activated cell sorter, utilising monoclonal antibodies.[3] The buffy coat from fresh heparinised venous blood was incubated with titrated mouse monoclonal antibody, while control cells were incubated with non-specific mouse IgG. After lysis of the red cells, the white cell pellet was washed and incubated with FITC goat F $(ab')_2$ anti-mouse IgG (Cappel Laboratory, Cochranville, PA) for 45 minutes at 4°C, and washed again.

Flow cytometry was performed on a cell sorter equipped with an argon ion laser, operated at a 488 nm wavelength and 400 mW for fluorescein excitation (FACS IV, Becton-Dickinson, Sunnyvale, CA or Cytofluorograph 50 H, Ortho Pharmaceuticals, Raritan, NJ). Samples were analysed for forward light scatter, right angle light scatter and fluorescence. White blood cells were counted with a Coulter ZBI. Leukocyte differentials were determined by counting 100 cells on a Giemsa-stained blood smear. Groups were statistically compared using the Student's t-test.

Validation of lymphocyte analy-

*From the Pulmonary Section, USC School of Medicine, Los Angeles, California, U.S.A.

sis by flow cytometry was conducted by staining with OKM-1 monoclonal antibody, which identified monocytes and polymorphonuclear leukocytes.

Processing of lung biopsy tissue

Tissues were rapidly frozen in liquid nitrogen after being placed in OCT compound (embedding media for frozen tissue specimens, Lab-Tek Products, Miles Laboratories Inc., Kankakee, IL.), and were then stored at -70°C until sectioned. Lymphocyte antigens were detected by a modified immunoperoxidase method.[4,5] The primary monoclonal antibodies used were directed against a pan T-cell marker (Leu 1, Becton-Dickinson), a T-helper/inducer marker (Leu 3) and a T-suppressor/cytotoxic marker (Leu 2).

RESULTS

Bronchoalveolar lavage (BAL)

There were six lavage fluids from six sarcoidosis patients and three from the control subjects. All patients with sarcoidosis showed anticipated lymphocytosis. The helper/inducer to suppressor/cytotoxic T-cell ratio was high in bronchial washings but it was decreased in the peripheral blood (Table 1).

Table 1 Helper/suppressor ratio in peripheral blood and bronchoalveolar lavage fluid of six patients with active sarcoidosis

Subjects (n)	Helper/Suppressor Ratio		Serum ACE (units)
	Blood	BAL fluid	
Control (3)	0.9 – 2.9	0.9 – 2.9	10 – 30
Sarcoidosis (6)	0.3 – 1.0 (Mean 0.48)	8.9 – 11 (Mean 9.8)	39 – 84 (Mean 54)

ACE = Angiotensin converting enzyme

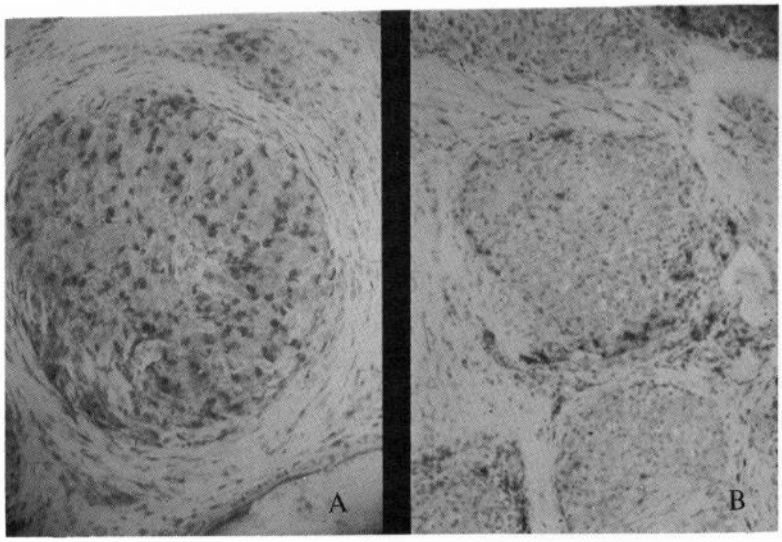

Fig. 1 A. Showing numerous helper/inducer cells distributed throughout the granuloma. (Frozen section, Leu 3, no counterstain, x 130).

B. Showing suppressor/cytotoxic cells encircling the granulomas (Frozen section, Leu 2, no counterstain, x 80).

Lung biopsy specimens

The lung biopsy specimens showed circumscribed epithelioid cell granulomata surrounded by a sparse mantle of lymphocytes. With immunoperoxidase staining, lymphocytes bearing the pan T-cell antigen were found to be present throughout the mantle but less densely within the epithelioid cell aggregates. Lymphocytes expressing the T-suppressor/cytotoxic phenotype were localised predominantly in the mantle (Figs. 1 A. and B), and only rarely within the granulomata. In contrast, lymphocytes expressing the T-helper/inducer antigenic determinant were present throughout the epithelioid cell aggregates and showed no predilection for the mantle. The ratio of helper-to-suppressor phenotypes was greater than 5:1.

DISCUSSION

Immunohistochemical staining of sarcoid granulomata showed the central localisation of cells expressing T-helper/inducer phenotypes. These cells were distributed among the aggregated epithelioid cells, whereas cells bearing the suppressor/cytotoxic phenotype were restricted to the peripheral region. Perhaps this intimate admixture of helper T cells and epithelial histiocytes is effective in mounting a granulomatous response. At the same time, the suppressor/cytotoxic cells, due to their localisation, may help to contain this immunological response. It is conceivable that sequestration of helper T cells in diseased tissues may account for the low helper-to-suppressor T-cell ratio in the peripheral blood of our sarcoidosis patients. These results are in accord with the report of Hunninghake and Crystal[5] who found a similarly high ratio in bronchial washings.

This study has extended the observation of Semenzato *et al*[2] who demonstrated this peculiar localisation of suppressor/cytotoxic cells to the mantle surrounding the granuloma in skin, lung and lymph node biopsies.

The segregation of helper and suppressor T cells in sarcoidosis is

similar to the distribution observed in tuberculoid leprosy, but different from that seen in lepromatous leprosy. The striking consistency of patterns as they occur within these different conditions, and their relationship to immune reactivity, suggest that the localisation of T-cell subpopulations and their role in immune competence needs further study.

REFERENCES

1. Sharma OP. Sacoidosis: clinical management. London: Butterworths, 1984.
2. Semenzato G, Pezzutto A, Chilosi M, *et al.* Redistribution of T lymphocytes in the lymph nodes of patients with sarcoidosis. N Engl J Med 1982; 306:48-9.
3. Modlin RL, Meyer RR, Hofman FM, *et al.* In situ demonstrations of T-lymphocyte subsets in leprosy, rhinoscleroma and sarcoidosis. Clin Exp Immunol 1983; 51:430-8.
4. Modlin RL, Hofman FM, Sharma OP, *et al.* Demonstration in situ of subset of T-lymphocytes in sarcoidosis. Am J Dermatopathol 1984; 6:423-7.
5. Hunninghake GW, Crystal RG. Pulmonary Sarcoidosis: a disorder mediated by excess helper T-lymphocyte activity at sites of disease activity. N Engl J Med 1981; 305: 429-34.
6. Modlin RL, Gebhard JF, Taylor CR, *et al.* In situ characterization of T-lymphocyte subsets in the reactional states of leprosy. Clin Exp Immunol 1983; 53:17-24.