

Total T lymphocytes in primary bronchial carcinoma

H. L. ROBERTS, W. T. A. DONOHOE, SHEILA HEWITT, AND D. A. PRICE EVANS

From the Department of Medicine, University of Liverpool, Liverpool L69 3BX

Roberts, H. L., Donohoe, W. T. A., Hewitt, Sheila, and Price Evans, D. A. (1977). Thorax, 32, 84-87. Total T lymphocytes in primary bronchial carcinoma. Percentage and absolute levels of circulating T lymphocytes were measured in 48 patients with bronchial carcinoma. These were compared with control values from nine healthy adults and 19 age-matched patients with benign disorders. A further 20 patients who had been given postoperative immunotherapy after complete resection of bronchial carcinoma were also studied. There was no significant difference in the mean percentage T cells between the groups. Lymphopenia, however, was a feature of the bronchial cancer patients with metastatic disease. This resulted in a significant diminution of absolute T cells in this group. There is no evidence, with the technique employed in this study, of a total T-cell deficiency in early bronchial carcinoma.

There is increasing interest in the immunological aspects of neoplasia in both reticuloendothelial and solid tumours. Inefficiency of the immune surveillance mechanism, allowing the proliferation of clones of abnormal cells, has been suggested as an aetiological factor in the development of cancer (Burnet, 1970). Neoplasia, especially in its more advanced stages, is associated with a diminution of the cell-mediated immune response. The fundamental question, however, whether the immunological deficiency precedes the development of the tumour or is secondary to the presence of the tumour, remains unanswered.

At least two functionally distinct populations of small lymphocytes have been recognised (Greaves *et al.*, 1974). T lymphocytes are concerned with the cell-mediated immune function while B lymphocytes are involved in humoral antibody production. Numerous studies have been reported on lymphocyte subpopulations in patients with various solid tumours (Catalona *et al.*, 1974; Wanebo *et al.*, 1975; Whitehead *et al.*, 1976), including bronchial carcinoma (Anthony *et al.*, 1975; Dellon *et al.*, 1975; Gross *et al.*, 1975). Depression of T lymphocyte levels have been described in both early and late stages of the disease. Measurement of circulating T cells consequently deserved consideration as a prognostic index for use during immunotherapy for bronchial carcinoma. The aim of the present study was to de-

termine the circulating T lymphocyte levels in patients with primary bronchial carcinoma at various stages and to compare the values with those obtained in subjects without cancer.

Patients and methods

Ninety-six individuals were studied—9 healthy controls, 19 patients with benign disease (hernia, cholelithiasis, diverticulosis coli), and 68 bronchial carcinoma patients. Of the cancer patients, 25 had disease confined to lung parenchyma or hilar nodes ('localised' disease); staging was assessed as a result of exploratory thoracotomy but the T cell studies had been carried out before this thoracotomy. A further 25 patients had evidence of extension of tumour to mediastinal lymph nodes or bloodborne distant metastases ('metastatic disease'). Histological confirmation was obtained in all of the localised cases, but seven of the metastatic disease cases were diagnosed and staged clinically without thoracotomy or histological evidence. Twenty postoperative patients who had received immunotherapy were also studied (see Disease-free column, Table 1). These immunotherapy patients had been randomised into two treatment groups, (1) BCG plus adjuvant (at separate sites) and (2) BCG plus adjuvant mixed with irradiated autochthonous tumour cells (at separate sites). The status of individuals (i-

whether (1) or (2)) remains unknown to the experimenters. The mean age of the patients with benign disease was similar to the mean age of the cancer patients.

In view of the known factors depressing *in vitro* assays of cellular immunity, patients were excluded if they had received chemotherapy, steroids or radiotherapy (Catalona *et al.*, 1974) in the 18 months before testing. Patients were not studied within seven days of receiving a general anaesthetic or within one month of a blood transfusion.

Tests were arranged so that on each day one or two samples from the non-cancer groups were processed concurrently with blood samples from the lung cancer patients.

LYMPHOCYTE SEPARATION

Lymphocytes were initially isolated from sequestered venous blood by layering 3.5 ml of blood diluted with an equal volume of phosphate buffered saline (PBS) over 5.5 ml of Ficol-Hypaque mixture of SG 1078 (Thorsby and Bratlie, 1970). This was then centrifuged at 400 *g* for 40 minutes at 4°C. Interface cells, however, were often found to contain a significant proportion of neutrophils, erythrocytes, and platelets, rendering accurate rosette counting difficult and time-consuming.

The preparation was, therefore, modified by the use of carbonyl iron (Doughty and Gelsthorpe, 1971) to remove the unwanted cells. The method briefly consisted of incubating fresh sequestered venous blood for 30 minutes at 37°C with an equal volume of carbonyl iron preparation. The bulk of neutrophils, erythrocytes, and platelets sediment with the iron particles by gravity. The resulting cloudy supernatant was then carefully layered onto Ficol-Hypaque SG 1078 and centrifuged at 1300 *g* for 10 minutes. The interface cells, consisting of a pure mononuclear preparation, were washed twice in PBS and adjusted to 7×10^6 ml.

E ROSETTE ASSAY

The rosetting technique of Anthony *et al.* (1975)

was followed with minor modifications. Lymphocyte suspension, 0.1 ml, was added to 0.1 ml of thrice-washed sheep red blood cells (SRBC) at 1/50. The mixture was incubated at 37°C for 10 minutes, gently centrifuged at 90 *g* for five minutes, and left overnight in an ice-bath. The cells and rosettes were then resuspended by rotation at 24 rpm for two minutes on a disc rotator, 25.4 cm in diameter. One drop of the mixture was carefully added to one drop of trypan blue and mounted on a chilled haemocytometer chamber. Tubes were run in duplicate. At least 600 cells were counted for each preparation, all counts being made without knowledge of the patient's identity. Rosettes were defined as lymphocytes with three or more SRBC but most rosettes were complete morulas.

The absolute T count was estimated by the formula
total leucocyte count \times % lymphocytes \times % T cells.

Results

The mean percentage and absolute T lymphocyte counts for each group are shown in Table 1. There was no significant difference in T-cell percentages in any of the groups. Lymphopenia was, however, found in patients with advanced cancer, and this resulted in a significantly lowered absolute T-cell count ($P < 0.001$ by Scheffé (1953) test) (Figure). The seven patients with metastatic disease, whose histological diagnosis was not known, had similar values to the other 18 in whom the histology was known.

In view of the small numbers, analysis of each histological type was not performed. As might be expected, there were far fewer cases of oat-cell tumour in the 'localised' disease category (1 out of 23) than in the metastatic group (7 out of 18) (see Table 2).

Discussion

Differing patterns of immunological deficiency are found in patients with cancer. The clearest

Table 1 Rosette formation with lymphocytes from bronchial carcinoma

	Non-cancer subjects		Bronchial carcinoma subjects		
	Healthy adults	Benign disease	Localised	Metastatic	Disease-free
No. of patients	9	19	23	25	20
Age. Mean \pm SD	31.3 \pm 13.7	57.7 \pm 10.4	61.1 \pm 7.1	61.2 \pm 6.4	60.7 \pm 5.8
Absolute lymphocyte count. Mean \pm SD	2253 \pm 706	2214 \pm 777	2411 \pm 750	1461 \pm 664	2426 \pm 811
T cells %. Mean \pm SD	66.8 \pm 4.4	68.2 \pm 7.5	66.7 \pm 8.7	64.4 \pm 13.4	67.1 \pm 9.8
Absolute T-cell count. Mean \pm SD	1478 \pm 460	1496 \pm 495	1624 \pm 571	970 \pm 556	1674 \pm 729

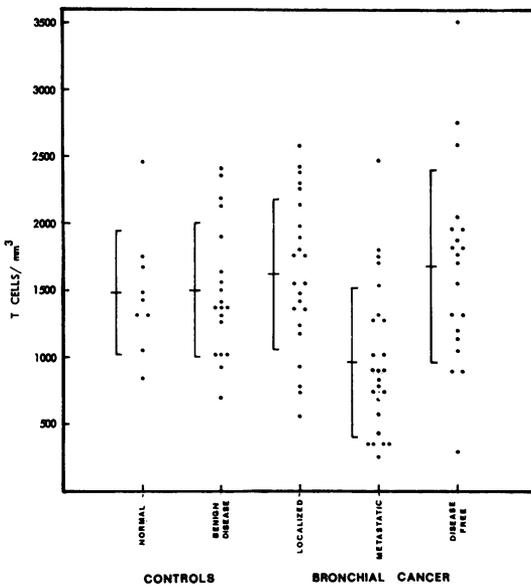


Figure Total T cells in bronchial carcinoma (mean \pm 1SD).

Table 2 Histological type of bronchial carcinoma patients

Histology	Local	Metastatic	Disease-free
Squamous	14	6	11
Oat cell	1	7	4
Adenocarcinoma	5	2	5
Undifferentiated	3	3	—
Totals	23	18	20

evidence (and most published literature) is concerned with cancer involving the immune system itself. Hodgkin's disease shows a deficiency of T-cell functions in all stages (Aisenberg, 1976). In the more common cancers, the situation is not so clearcut. Head and neck cancers demonstrate immunological deficiencies early in the course of the disease (Wanebo *et al.*, 1975) and these get worse as the disease progresses. In women with breast cancer, generalised immune deficiency becomes pronounced only with advanced disease, but T lymphocyte levels are diminished in the early stages (Whitehead *et al.*, 1976). In lung cancer, studies have shown cell-mediated immune deficiency at various stages of the disease (Krant *et al.*, 1968; Brugarolas *et al.*, 1973; Dellon *et al.*, 1975).

The finding in the present research of absence of depression of T cells in both proportional and absolute terms in localised primary lung cancer

is not in accord with most other studies (Anthony *et al.*, 1975; Dellon *et al.*, 1975; Gross *et al.*, 1975). Dellon *et al.* (1975), in a large series of patients (using a similar rosette-forming technique to that employed in the present study but without a carbonyl iron step), showed that T-cell levels in preoperative squamous, oat-cell, and undifferentiated carcinoma correlated well with the extent of the tumour. Even localised disease showed a significant diminution in absolute T-cell levels compared to normals.

Our findings of normal T lymphocyte levels in early disease and of lymphopenia with advanced carcinoma are, however, similar to functional lymphocyte studies performed by Barnes *et al.* (1975). These authors found that phytohaemagglutinin (PHA) responsiveness of peripheral lymphocytes of newly presenting patients with lung cancer showed no difference from healthy age- and sex-matched normal controls. There was, however, a significant diminution in PHA response in the preterminal phase of the disease. Harris *et al.* (1975), in a study of a variety of disseminated solid tumours (but excluding lung cancer), found the T-cell count of such patients different from that of healthy control subjects. Their technique differed in detail from that employed in the present study, for example, by the preliminary exposure of the lymphocytes to fetal calf serum. Similar results of both T-cell number and function in bronchial carcinoma have been found by Amlott and Knight (personal communication).

The literature on T-cell estimation is unfortunately plagued by a variety of differing techniques. Our present study adds yet another method but we feel justified in its use in eliminating neutrophils and thereby increasing the efficiency and accuracy of the counting procedure.

The technique described with its long incubation period measures the total T-cell count in peripheral blood. It may well be that only a proportion of the T cells are concerned with tumour immune recognition, and alternative techniques are necessary to define such a subpopulation. Fudenberg *et al.* (1975), using the 'active' rosette test, ie, allowing the SRBC and lymphocytes to incubate for a short time, claim that significant differences occur between healthy controls and early cancer patients.

Our observations suggest that a primary depletion of the total T lymphocyte population preceding the development of lung cancer does not occur. A more sensitive test of a T lymphocyte subpopulation may show a difference in the early

Thorax 1977; 32: 85-88

cancer patient. Depression of total T cells seems to follow the development of metastatic disease.

We wish to thank The Cancer Research Campaign and the North West Cancer Research Fund for financial support, and the staff of the Thoracic Surgical Units at Broadgreen and Fazakerley Hospitals, Liverpool for access to patients.

References

- Aisenberg, A. C. (1976). In *Scientific Foundations of Oncology*, edited by T. Symington and R. L. Carter, p. 539. Heinemann, London.
- Anthony, H. M., Kirk, J. A., Madsen, K. E., Mason, M. K., and Templeman, G. H. (1975). E and EAC rosetting lymphocytes in patients with carcinoma of bronchus. 1. Some parameters of the test and of its prognostic significance. *Clinical and Experimental Immunology*, **20**, 29-40.
- Barnes, E. W., Farmer, A., Penhale, W. J., Irvine, W. J., Roscoe, P., and Horne, N. W. (1975). Phytohemagglutinin-induced lymphocyte transformation in newly presenting patients with primary carcinoma of the lung. *Cancer*, **36**, 187-193.
- Brugarolas, A., Han, T., Takita, H., and Minowada, J. (1973). Immunologic assays in lung cancer. *New York State Journal of Medicine*, **73**, 747-750.
- Burnet, F. M. (1970). The concept of immunological surveillance. *Progress in Experimental Tumour Research*, **13**, 1-27.
- Catalona, W. J., Potvin, C., and Chretien, P. B. (1974). Effect of radiation therapy for urologic cancer on circulating thymus-derived lymphocytes. *Journal of Urology*, **112**, 261-267.
- Dellon, A. L., Potvin, C., and Chretien, P. B. (1975). Thymus-dependent lymphocyte levels in bronchogenic carcinoma: Correlations with histology, clinical stage, and clinical course after surgical treatment. *Cancer*, **35**, 687-694.
- Doughty, R. W. and Gelsthorpe, K. (1971). A scheme for screening blood samples to acquire lymphocyte cytotoxic antisera for tissue typing and results of an initial screening programme. *Transplantation*, **12**, 429-433.
- Fudenberg, H. H., Wybran, J., and Robbins, D. (1975). T-rossette-forming cells, cellular immunity and cancer. *New England Journal of Medicine*, **292**, 475-476.
- Greaves, M. F., Owen, J. J. T., and Raff, M. C. (1974). *T and B Lymphocytes: Origins, Properties and Roles in Immune Responses*. Excerpta Medica, Amsterdam, p. 81.
- Gross, R. L., Latty, A., Williams, E. A., and Newberne, P. M. (1975). Abnormal spontaneous rosette formation and rosette inhibition in lung carcinoma. *New England Journal of Medicine*, **292**, 439-442.
- Harris, J., Stewart, T., Sengar, D. P. S., and Hyslop, D. (1975). Quantitation of T- and B-lymphocytes in peripheral blood of patients with solid tumours. I. Relation to other parameters of *in vivo* and *in vitro* immune competence. *Canadian Medical Association Journal*, **112**, 948-952.
- Krant, M. J., Manskopf, G., Brandrup, C. S., and Madoff, M. A. (1968). Immunologic alterations in bronchogenic cancer. *Cancer*, **21**, 623-631.
- Scheffé, H. (1953). A method for judging all contrasts in the analysis of variance. *Biometrika*, **40**, 87-104.
- Thorsby, E. and Bratlie, A. (1970). A rapid method for preparation of pure lymphocyte suspensions. In *Histocompatibility Testing*, edited by P. I. Terasaki, pp. 665-666. Munksgaard, Copenhagen.
- Wanebo, H. J., Jun, M. Y., Strong, E. W., and Oettgen, H. (1975). T cell deficiency in patients with squamous carcinoma of the head and neck. *American Journal of Surgery*, **130**, 445-451.
- Whitehead, R. H., Thatcher, J., Teasdale, C., Roberts, G. P., and Hughes, L. E. (1976). T and B lymphocytes in breast cancer stage relationship and abrogation of T-lymphocyte depression by enzyme treatment *in vitro*. *Lancet*, **1**, 330-333.

Requests for reprints to: Dr. H. L. Roberts, Department of Medicine, University of Liverpool L69 3BX.