

Lymphocyte Subpopulations in Peripheral Blood and Malignant Effusions of Cancer Patients

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Abstract—The percentage of total T lymphocytes and helper and suppressor cells was determined in the peripheral blood and pleural and abdominal effusions of patients with advanced cancer. The ratio of helper to suppressor cells in the peripheral blood and in the effusions was calculated in each case. The percentage of total T lymphocytes and suppressor cells was found to be in a normal range. The percentage of helper cells in patients' peripheral blood and in ascites was slightly lower than normal. However, the percentage of helper cells in the pleural effusion was significantly higher than in the blood. The ratio of helper to suppressor cells in the pleural effusion was 1.46, compared to 0.82 in the blood ($P < 0.01$). In patients with ascites the ratio in the blood was 1.05 and in the effusion 1.28. The ratio of helper to suppressor cells of all patients in the peripheral blood was 0.94, as compared to 1.36 in the effusion fluid ($P < 0.05$). The imbalance in the T cell subsets found is most probably due to immunodepressions in the peripheral blood and immune reactivity in the effusions.

INTRODUCTION

IMPAIRMENT of cell mediated immunity has been shown by many authors, including ourselves, to correlate with advanced stage of malignant neoplasia [1, 2].

Several recent studies have reported on the percentage of helper and suppressor cells and the ratio between them in the peripheral blood of cancer patients [3-8]. The results show that in localized cancer and in malignant melanoma there is a no-systemic imbalance in the T cell subsets. In advanced cancer there is an imbalance in which the normal 2:1 ratio of helper to suppressor cells is altered due to reduction in the percentage of helper cells.

As far as we are aware, no data have been published concerning T cell subsets populations in the pleural or abdominal effusions of cancer patients. The purpose of the present study was to determine the percentage of helper and suppressor cells, and the ratio between them in the peripheral blood and pleural and abdominal effusions of the cancer patients.

MATERIALS AND METHODS

Nineteen patients with metastatic cancer were studied: four ovarian carcinoma, nine breast cancer, one pancreas carcinoma, one colon carcinoma

and four metastatic cancer of unknown primary origin. All had radio- and/or chemotherapy. This treatment was stopped at least 1 month before testing. Some patients were still on hormonotherapy. Pleural fluid was tested in nine cases, ascitic fluid in ten. Tumour cells were found in all effusions. Peripheral blood from each patient was tested on the same day as the effusion. The monoclonal study was carried out as described by Reinherz *et al.* [9]. Heparinized blood (5 ml), or pleural or abdominal effusions (400 ml) were withdrawn. Mononuclear cells were obtained using the standard fractionation Ficoll-Hypaque gradient centrifugation technique by Boyum [10].

Helper and suppressor T cells were identified by employing three murine monoclonal antibodies obtained from Ortho Pharmaceuticals, Raritan, NJ: OKT₃ for mature T lymphocytes, OKT₄ for helper T cells and OKT₈ for suppressor T cells. The appropriate monoclonal antibody was added to aliquots of $1-2 \times 10^6$ cells, and the cells were stained by an immunofluorescent technique using a fluorescein-labelled goat anti-mouse IgG [FITC Conj. goat anti-mouse IgG antibody (Gamma Light ch. op.), Tago Inc., Burlingame, CA].

Counts were made of at least 200 cells using a microscope equipped for alternating phase contrast, bright field and fluorescence examinations.

RESULTS

Table 1 records the mean percentages of total T

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cells and the two T cell subpopulations, as well as the ratio of helper to suppressor cells in the ascites and in the peripheral blood. The percentage of helper cells was slightly lower than normal in blood and in the ascites, and did not differ from the percentage of the suppressor cells. No significant difference was found between the corresponding values of total T cells and suppressor cells in the ascites and blood. The ratio of helper to suppressor cells in the blood was 1.05 and in the ascites 1.28.

Table 2 summarizes the mean percentages of total T cells and the two T cell subpopulations as well as the ratio of helper to suppressor cells in pleural fluids and in the peripheral blood. Again the percentage of helper cell in the blood was lower than the normal. The difference between the percentage of helper T cells (53.1% in the fluid vs 27.2% in the blood) was statistically significant ($P < 0.01$). In consequence a significant difference was found between the ratio of helper/suppressor cells in the fluid (1.46) and that in the blood (0.82) ($P < 0.01$). The difference between the T cell subsets of abdominal and pleural effusions was not significant.

Table 3 summarizes the results of the distribution of T cell subsets, and the ratios between them in both pleural and abdominal effusions and their corresponding blood samples. The mean total T content of the blood was 52.69% and the mean of the ratios of helper/suppressor cells was 0.94. In the effusions the total T was 55.75% and the mean of the H/S ratios was 1.36.

On average the percentages of total T and suppressor T cells in the peripheral blood did not differ significantly from their corresponding percentages in the effusions. However, the average of helper T cells in the effusions was significantly higher than in the blood ($P < 0.02$), and the mean of the ratios of helper to suppressor cells was also significantly higher in the effusion 1.36 than in the blood 0.94 ($P < 0.05$).

DISCUSSION

The percentages of total T and suppressor T cells in the peripheral blood found in this study are similar to those recorded for normal healthy subjects [9]. However, a relatively lower mean percentage of helper T cells was found. In a study on the distribution of T cell subsets in the peripheral blood of melanoma patients, Werkmeister *et al.* found that the ratio of mean helper to mean suppressor cells in the blood was 2:1 [8].

Similarly, Bernengo *et al.* found in melanoma patients a ratio of 2:1.3 helper to suppressor cells [3]. Karavodin *et al.* [5] did not find any imbalance in T cell subsets in melanoma. McCluskey *et al.* [7] studied the total T lymphocyte and lymphocyte subset populations in patients with breast cancer. Patients in whom the cancer was localized displayed a total T cell population and a ratio of helper to suppressor cells similar to those in normal controls. However, patients with advanced disease showed a reduction in helper cell population and the ratio of helper/suppressor cells was 0.6, lower than our

Table 1. Lymphocyte subpopulations in peripheral blood and ascites

	Total T lymphocytes (OKT ₃)	Helper T cells (OKT ₄)	Suppressor T cells (OKT ₈)	Ratio H/S
Blood	49.50 ± 6.73	32.33 ± 5.60	32.40 ± 4.84	1.05 ± 0.18
Ascites	55.90 ± 4.65	38.03 ± 5.53	39.04 ± 5.66	1.28 ± 0.22
Statistical evaluation	N.S.	N.S.	N.S.	N.S.

Mean percentage of lymphocytes ± S.E. H/S, helper to suppressor cell.

Table 2. Lymphocyte subpopulations in pleural effusions and in peripheral blood

	Total T lymphocytes (OKT ₃)	Helper T cells (OKT ₄)	Suppressor T cells (OKT ₈)	Ratio H/S
Blood	55.86 ± 4.53	27.20 ± 4.03	33.90 ± 4.03	0.82 ± 0.13
Pleural	50.76 ± 6.11	53.10 ± 6.43	35.30 ± 4.18	1.46 ± 0.12
Statistical evaluation	N.S.	P < 0.01	N.S.	P < 0.01

Mean percentage of lymphocytes ± S.E.

Table 3. Lymphocyte subpopulations in abdominal and pleural effusions and in peripheral blood

	Total T lymphocytes (OKT ₃)	Helper T cells (OKT ₄)	Suppressor T cells (OKT ₈)	Ratio H/S
Blood	52.69 ± 4.05	29.9 ± 3.81	33.16 ± 3.11	0.94 ± 0.11
Abdominal and pleural fluid	55.76 ± 3.2	44.73 ± 4.45	37.26 ± 3.51	1.36 ± 0.14
Statistical evaluation	N.S.	<i>P</i> < 0.02	N.S.	<i>P</i> < 0.05

Mean percentage of lymphocytes ± S.E.

finding of 0.94. In the effusions of our patients the ratio of mean helper to mean suppressor cells was significantly higher (*P* < 0.01) than the peripheral blood — 1.36 vs 0.94.

In a recent study of T lymphocyte subpopulations in patients with Sarcoidosis, Groman *et al.* [11] found that the ratio of helper to suppressor cells was 0.8 in peripheral blood and 4.2 in the pleural fluid, the latter result being due mainly to an increase in the mean percentage of helper cells. These findings are similar to ours. The reason for the present finding of a low mean value of helper T cells in the blood of cancer patients and the higher

values in the pleural fluid is not clear. The immunodepression found in patients with advanced cancer is probably due to a decrease in helper cells. In patients with melanoma the increase in the helper cell subpopulation could be a sign of immune reactivity. The increase in helper cell content in the pleural effusion fluid may be an indication of host reactivity against the tumour cell. The reason why this occurs only in pleural fluids and not in abdominal effusion is not clear. Further studies are being carried out in our laboratory to clarify this point.

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