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Changes of Blood T Cell Subsets in Patients Receiving Postoperative Adjuvant Chemotherapy for Breast Cancer*

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Abstract—*The number of T helper (T_h) and T suppressor (T_S) cells, as defined by monoclonal antibodies, of the blood lymphocyte population was examined in breast cancer patients receiving postoperative cyclic therapy with cyclophosphamide, methotrexate and 5-fluorouracil. The total number of lymphocytes was reduced to approximately* 50% *at the end of the* 17-*month period of chemotherapy. Identification of T_h and T_S subsets with the aid of Leu-3a and Leu-2a antibodies revealed that the former was reduced to a higher relative extent than the latter, thus reducing the T_h/T_S ratio highly significantly during the entire treatment period. A reduced ratio was also observed in a group of patients having completed their treatment* 2-3 yr *earlier.*

INTRODUCTION

CYCLIC chemotherapy was recently introduced as an adjunct to surgery in patients with relatively advanced breast cancer [1]. This type of therapy has been shown to depress both T and non-T lymphocyte counts and several immune functions *in vitro* [2]. On the other hand, certain cytotoxic functions may be increased [3].

We have previously reported that T lymphocytes with both 'helper' or 'suppressor' phenotypes were reduced after radiation therapy for breast cancer [4]. Until now we have only reported preliminary data on the effect of modern cyclic chemotherapy for breast cancer on these subpopulations [5]. In the present investigation we have determined with the aid of monoclonal antibodies the size of T helper and T suppressor subsets in the blood during and after adjuvant cyclic therapy of breast cancer patients with cyclophosphamide, methotrexate and 5-fluorouracil (CMF).

MATERIAL AND METHODS

Patients and controls

Thirty-four women with microscopically confirmed primary mammary carcinoma are presented. Their ages ranged from 40 to 69 yr, with a mean of 53 yr. All patients had been operated on, the primary tumour exceeded 3 cm in diameter and/or the axillary lymph nodes were involved. Twenty-three healthy women 36-65 yr of age (mean 50 yr) served as controls.

Treatment

The patients had undergone a modified radical mastectomy with axillary dissection. Cyclic CMF chemotherapy was started 4-6 weeks later. The patients received 12 cycles, each consisting of 40 mg/m² of methotrexate and 600 mg/m² of 5-fluorouracil i.v. on days 1 and 8 and 100 mg/m² of cyclophosphamide by mouth on days 1-14. The next cycle was started on day 42. Doses were reduced in one or several cycles because of side-effects, mainly myelotoxicity, as described previously [2].

Blood sampling

Peripheral lymphocytes were examined postoperatively before the first cycle and before cycle Nos III, V, VII, IX and XII. Subsequent samples were obtained 3 and 6 months later. If distant metastases were detected the patient was deleted from further study. Another group of 12 patients was examined 2-3 yr following CMF therapy to

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study long-term effects of treatment. Pretreatment values were not available in these patients.

Determination of lymphocyte numbers

Numbers of leucocytes per unit of blood were examined by Coulter counter, and differential counts were made on May-Grünwald-Giemsa smears.

Examination of lymphocyte subpopulations

Lymphoid cells were separated from heparinized venous blood by centrifugation on Ficoll-Isopaque and phagocytic cells were removed by a magnet [6]. T lymphocyte subsets reacting with fluoresceinisothiocyanate-conjugated monoclonal antibodies against helper T (T_h) and suppressor T (T_s) cell antigens (anti-Leu-3a and anti-Leu-2a, respectively, Becton Dickinson) were determined by immunofluorescence [4, 7].

Data processing and statistical analysis

The initial value of various lymphocyte counts of each patient was put as 100% and the subsequent values were related to this. Geometrical means ± S.E. of these relative values were calculated. T_h/T_s ratios were calculated on an arithmetic basis. Statistical differences between means were calculated using Student's *t* test.

RESULTS

The pretreatment frequencies of T_h and T_s cells in the blood of patients and healthy controls are listed in Table 1. There were no differences between the two groups of individuals.

The changes in total lymphocyte counts are shown in Fig. 1. The total number was gradually reduced during the course of the treatment and reached a minimum of approximately 50% before cycle No. XII. When treatment was completed this reduction seemed to be followed by a slight recovery. The number of T_h cells was reduced in a similar way and reached its nadir of approximately 40% at the last cycle. On the other hand, the number of T_s cells was less affected by the treatment, with a nadir of 70%. Statistically significant reductions of this subset were only noted on two occasions during the treatment (Fig. 1).

Table 1. Frequencies of blood T lymphocyte subsets in breast cancer patients and in healthy controls (means ± S.E.)

	Patients before CMF (n = 23)	Controls (n = 23)
T_h cells (%)	52 ± 3	52 ± 3
T_s cells (%)	25 ± 2	24 ± 2

The differential depletions of T_h and T_s cells became even more prominent when the ratio between the subsets was examined (Fig. 2). The ratio fell from approximately 2.5 to around 1.5 before the third cycle of chemotherapy and remained at this level during the rest of the observation period. The T_h/T_s ratio of other breast cancer patients who were tested 2-3 yr after completion of chemotherapy was also significantly reduced (Fig. 2).

DISCUSSION

Differential depletions of T helper and T suppressor cells causing a reduced T_h/T_s ratio have been noted during various types of drug-induced immunosuppression [8]. We have examined during and following adjuvant chemotherapy (CMF) for breast cancer changes of the two T lymphocyte subsets in the blood reacting

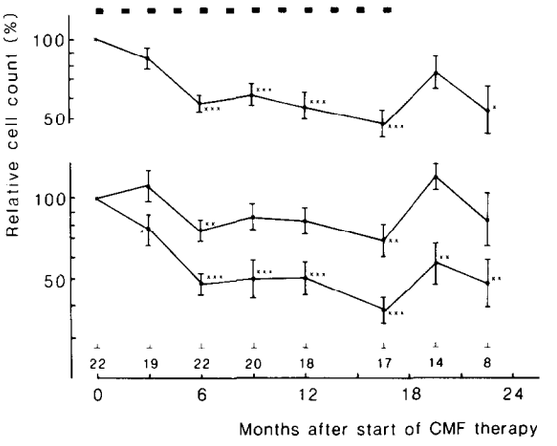

*Fig. 1. Relative changes of blood T lymphocyte subsets in cyclic chemotherapy (CMF). Top: total lymphocytes per μl. The initial log_{10} mean value ± S.E. was 3.20 ± 0.04 (geometrical mean = 1601). Middle: T_s cells per μl. The initial value was 2.58 ± 0.04 (geometric mean = 383). Bottom: T_h cells per μl. The initial value was 2.91 ± 0.05 (geometric mean = 803). Statistical differences compared to initial values: *P < 0.05, **P < 0.01, ***P < 0.001. Numbers of patients are given at bottom of diagram. Cycles of CMF are indicated by filled boxes.*

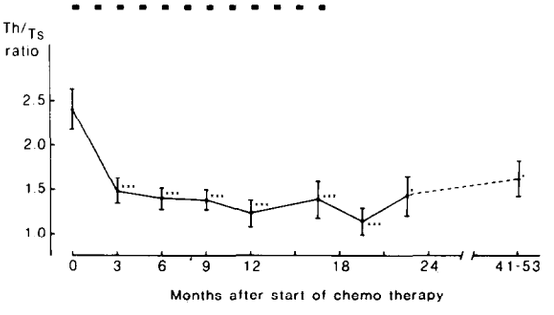

Fig. 2. Changes of T_h/T_s ratio in CMF treatment. Symbols as in Fig. 1.

with Leu-3a or Leu-2a monoclonal antibodies, considered to represent helper/inducer and suppressor/cytotoxic cells respectively [7].

We found that T_h cells were depleted to a higher extent than T_s cells (Figs 1 and 2). One explanation of this finding could be that T_h cells are more sensitive to the drugs used than T_s cells. Alternatively, the turnover of the T_s subset could be so high that it might be repopulated during the intervals between the CMF cycles. This view is supported by the previous finding that the T_s subset in the blood is restored faster than the T_h cells following radiation therapy of breast cancer patients [9], and that the Leu-2 subpopulation regenerated more rapidly after bone marrow transplantation than did the Leu-3 subpopulation, a decreased T_h/T_s ratio persisting for up to 1 yr after transplantation [10]. It is not known for how long the reduced T_h/T_s ratio persists after CMF therapy but the present results indicate no valid restitution 2-3 yr after finishing this treatment (Fig. 2). Reduced T_h/T_s ratios have been noted in patients successfully treated for Hodgkin's disease a long time after treatment with chemotherapy or radiation therapy [11] and up to a decade after radiation therapy for breast cancer [12]. Post-operative CMF chemotherapy is known to reduce the relative MLC- and PHA-reactivity, apart from reducing both T and non-T cells in the blood [2]. It is possible that the altered composition of the T cell population during and after CMF therapy may contribute to such functional changes of the lymphocyte population *in vitro* [3]. However, it is not known if it also alters the patients' defence against infectious agents.

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