Acta Radiologica Oncology 20 (1981) Fasc. 2

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ADJUVANT RADIATION THERAPY COMPARED WITH CYCLIC CHEMOTHERAPY IN PATIENTS WITH MAMMARY CARCINOMA

I. Changes of blood lymphocyte subpopulations

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Cyclic chemotherapy or radiation therapy may be valuable adjuncts to surgery in the treatment of relatively advanced breast carcinoma (BONADONNA et coll. 1976, WALLGREN et coll. 1978). The immediate and long term side effects of local radiation therapy on the peripheral lymphocyte population have been relatively well mapped both with regard to the sizes of various subpopulations and their immunologic functions (BLOMGREN et coll. 1974, CAMPBELL et coll. 1976, PETRINI et coll. 1977, BARAL et coll. 1977). The influence of postoperative cyclic chemotherapy on the blood lymphocyte compartments is as yet less completely known. Changes of various immunologic parameters during treatment with Methotrexate, 5-Fluorouracil and Chlorambucil were previously described (PETRINI et coll. 1979, STRENDER et coll. 1981). The patients have now been examined for 12 months after completion of cyclic chemotherapy with respect to changes of the sizes of blood lymphocyte subpopulations. For comparison similar tests were performed in parallel in patients given postoperative radiation therapy.

Materials and Methods

The series consisted of 37 women with microscopically confirmed primary breast carcinoma. A modified radical mastectomy including extirpation of axillary lymph nodes was performed in all the patients. If the primary tumor exceeded 3 cm in diameter or the axillary lymph nodes were involved, the patients were included in a clinical trial comparing postoperative prophylactic chemotherapy with adjuvant local radiation therapy. Nineteen patients (mean age 55, range 33–69 years) received postoperative cyclic chemotherapy and 18 (mean age 57, range 34–70 years) postoperative radiation therapy.

Chemotherapy. The first cycle of chemotherapy was started 4 to 6 weeks after surgery. Each cycle consisted of 600 mg/m^2 of 5-Fluorouracil and 50 mg of Methotrexate intravenously on day 1 and 8, and 15 mg of Chlorambucil by mouth on days 1 through 8. The next cycle started on day 42 in the majority of patients and 12 cycles were given. The doses of drugs were reduced in patients over 65 years and according to haematologic toxicity. The mean cumulative doses of 5-Fluorouracil and Methotrexate were 75 per cent (range 40–100%) and of Chlorambucil 85 per cent (range 55–100%) of the total doses initially planned. In 2 patients the

Accepted for publication 5 February 1981.

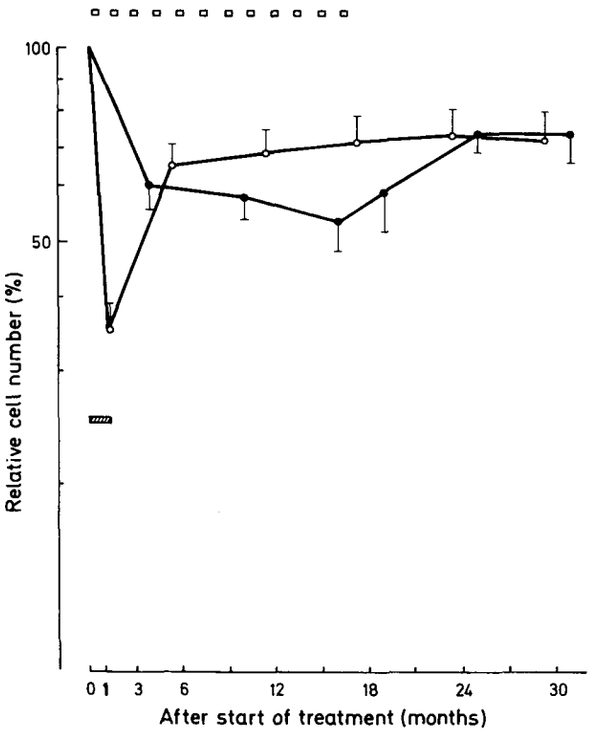

Fig. 1. Changes of blood lymphocyte counts in chemotherapy (●—●) or radiation therapy (○—○). Means and SE of 12–19 determinations. Periods of chemotherapy (□□), period of radiation therapy (▨).

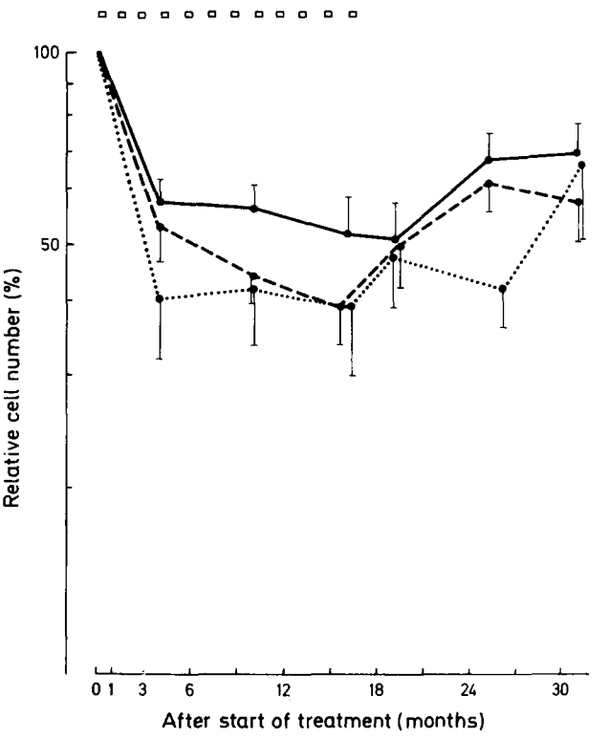

Fig. 2. Changes of E-cells (—), EAC-cells (---) and ME-cells (···) in chemotherapy. Means and SE of 11–17 determinations. Symbols as in Fig. 1.

last 4 cycles were given at intervals of 3 months. No correction has been made for this deviation from the treatment protocol since the values were similar to those of the rest of the patients.

Radiation therapy. Four to six weeks following surgery the operated area of the chest wall and the internal mammary, supraclavicular, and axillary regions were irradiated with 46 Gy (4 600 rad) using 6 to 8 MeV electrons and ^{60}Co gamma irradiation in 23 fractions during 5 weeks.

Blood sampling. Sample number I was taken immediately before start of adjuvant treatment. In the chemotherapy group samples number II, III, and IV were obtained on the same day that a new cycle was started; 4, 10, and 16 months after start of treatment. Samples V, VI, and VII were obtained 3, 9 and 15 months after its completion. In the radiation therapy group sample number II was obtained at completion of irradiation and number III 3 months later followed by samples number IV through VII with intervals of 6 months.

Separation and surface markers of lymphocytes. Lymphoid cells were separated from heparinized venous blood by Ficoll/Isopaque centrifugation followed by depletion of phagocytic cells and a second gradient centrifugation as described in detail previously (PETRINI et coll. 1977). Lymphocytes forming rosettes with sheep erythrocytes (E-cells), considered to be T cells, were determined according to the method of JONDAL et coll. (1972). Lymphocytes possessing membrane receptors for C'3 (EAC-cells), considered to be non-T cells, were determined as described previously (BLOMGREN et coll.). Lymphocytes forming rosettes with mouse erythrocytes (ME-cells), considered to be a population of B cells, were determined according to a slight modification (WASSERMAN et coll. 1978) of the original method described by GUPTA et coll. (1976).

Data processing and statistical method. The initial value of each patient was set as 100 per cent. The subsequent determinations of the same patient were related to this value and also expressed in per cent. Statistical differences between means were calculated using the Student's t-test.

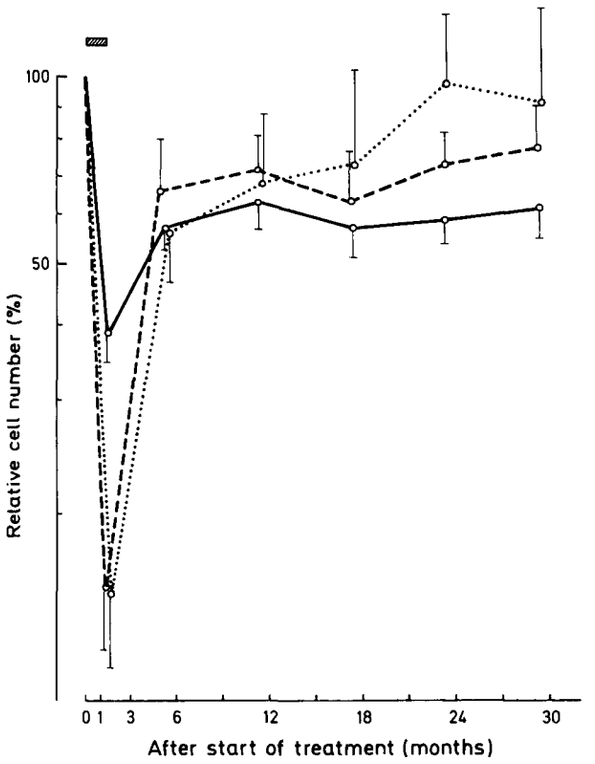

Fig. 3. Changes of E-, EAC-, and ME-cells following radiation therapy. Means and SE of 12–18 determinations. Symbols as in Fig. 2.

Table

Pretreatment counts of lymphocytes and lymphocyte subpopulations in patients with mammary carcinoma. Differences between means were not statistically significant. Means and SE. Number of tests in parentheses

	Chemotherapy	Radiation therapy
Lymphocytes (μl)	2 105±207 (19)	2 121±202 (18)
E-cells (μl)	1 297±143 (19)	1 286±130 (18)
EAC-cells (μl)	546±46 (17)	581±59 (14)
ME-cells (μl)	94±19 (17)	112±18 (18)

Results

Pretreatment total lymphocyte counts and the sizes of various subpopulations did not differ significantly between the two groups of patients (Table). The changes of total lymphocyte counts during and following treatment appear in Fig. 1. A progressive lymphopenia developed during chemotherapy and at the last cycle lymphocyte counts were reduced to 55 per cent followed by an increase to 70 per cent of the pretreatment level one year after termination of treatment.

The non-T lymphocyte populations, EAC- and ME-cells, were reduced to 40 per cent of the pretreatment level at completion of chemotherapy whereas the E-cell population was reduced to 50 per cent (Fig. 2). Thereafter all three subpopulations seemed to recover similarly thus creating a state of relative non-T cell deficiency during the year following chemotherapy.

Radiation therapy also reduced the non-T lymphocyte populations to the highest extent (Fig. 3). Both the EAC- and ME-cell populations were reduced to 15 per cent of pretreatment counts, whereas the E-cell population was decreased to 40 per cent. Both non-T cell populations recovered rapidly during the first 3 months after irradiation followed by a more retarded repopulation. The E-cell population recovered more slowly creating a state of relative T lymphocyte deficiency after irradiation.

Discussion

Radiation therapy significantly reduces the incidence of local recurrences in breast carcinoma (PATERSON & RUSSEL 1959, WALLGREN et coll.). However, this treatment may not reduce the development of distant metastases indicating that occult distant deposits of malignant cells are not affected. In an attempt to kill such cells several trials have been conducted in which high risk patients receive cyclic adjuvant chemotherapy postoperatively (BONADONNA et coll.). A randomized trial has been started in the Stockholm region to compare the clinical value of local radiation therapy with cyclic chemotherapy given postoperatively. When determining the clinical value of adjuvant chemotherapy for breast carcinoma it is necessary to take the side effects into account since some patients are permanently cured by surgery alone.

The aim of the present investigation was to compare the effects of local radiation therapy with adjuvant cyclic chemotherapy on the size and cellular composition of the peripheral lymphocyte populations. Although both types of treatment reduced both the T and non-T populations some interesting differences occurred. Radiation therapy reduced the non-T cell populations, as defined by EAC- and ME-rosette forming cells, to a higher extent than was observed during and at completion of

chemotherapy. However, repopulation of the non-T cell population proceeded more rapidly in the irradiated patients. It seems that the recovery of the T cell population after chemotherapy proceeded more rapidly than that of the non-T population.

The explanation of the rapid recovery of the non-T cell population in irradiated patients is not known. One possibility could be a migration of such cells into the blood from lymphoid organs which have not been irradiated. In the patients given chemotherapy, on the other hand, all lymphoid compartments including those of the blood and lymphoid organs were exposed to the cytotoxic drugs. Thus, the migration of lymphocytes from the latter compartments into the blood may have been impaired. Moreover, it is possible that non-T cells were damaged to a higher relative extent than T cells. This possibility is supported by the finding that treatment of mice with cyclophosphamide, an alkylating agent like Chlorambucil, which was given to the present patients, reduces the number of non-T cells in peripheral lymphoid tissues to a higher extent than T cells (POULTER & TURK 1972). In addition it has been shown that in vitro exposure of human peripheral lymphocytes to Melphalane or Adriamycin reduces the frequency of EAC-cells to a higher extent than E cells (BARAL et coll. 1980). Another explanation could be that the turnover of the non-T population, which has been shown to be higher than for the majority of T cells (RÖPKE & EVERETT 1973), was more permanently impaired by chemotherapy than by irradiation.

It is not known whether the changes of the blood lymphocyte populations induced by chemotherapy are of any disadvantage for the patients.

SUMMARY

Peripheral lymphocyte subpopulations were examined in 37 women with breast carcinoma who were postoperatively randominized to adjuvant local radiation therapy or cyclic treatment with 5-Fluorouracil, Methotrexate and Chlorambucil. Both T and non-T lymphocyte counts were reduced but the latter subpopulation was reduced to the highest relative extent in both groups of patients. Following radiation therapy the non-T cells repopulated more rapidly than the T cells, whereas the repopulation seemed to be the reverse in patients treated with chemotherapy.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Jerzy Einhorn for criticising the manuscript, and Mr Ricardo Giscombe, Miss Inger Vedin and Mrs Sirkka Nygren for excellent technical assistance. This investigation was supported by grants from King Gustaf Vth Jubilee Fund.

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