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T Lymphocyte Subpopulations in Blood Following Radiation Therapy for Breast Cancer*

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Abstract—*T helper and T suppressor lymphocyte subpopulations were examined before and after postoperative adjuvant radiation therapy for breast cancer. Absolute numbers of all T cell subsets were reduced by 70–80%. The proportion of IgG-Fc receptor-bearing T cells (T_g cells) was significantly reduced and that of IgM-Fc receptor-bearing T cells (T_m cells) significantly increased at completion of local radiation therapy with* 45 Gy (4500 rad). *Proportions of T suppressor (T_s) and T helper (T_h) cells determined by monoclonal antibodies were not changed by radiation therapy. The overlapping between T_g and T_s subpopulations was* 20–30% *as examined by double labelling.*

INTRODUCTION

IT HAS been previously shown that X-ray exposure of human blood T cell suspensions reduced the number of IgG-Fc receptor-bearing (T_g) cells to a higher extent than IgM-Fc receptor-bearing (T_m) cells [1]. T_g and T_m cells suppress and help, respectively, B cell responses in certain *in vitro* systems [2].

Recently introduced monoclonal antibodies (OKT 8 directed against antigens on suppressor/cytotoxic T cells and OKT 4 against helper/inducer T cells) provide another possibility to examine T cells with suppressor or helper functions. It was demonstrated that the radiosensitivity *in vitro* differed between suppressor cells defined by Fc receptors and by monoclonal antibodies [3], indicating that different cell populations might be involved.

In the present report we have examined T suppressor and T helper cell populations before and after radiation therapy for breast cancer by means of both Fc receptors and monoclonal antibodies. By double-labelling we also determined the extent of overlapping between T_g cells and T suppressor cells defined by monoclonal antibodies.

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MATERIALS AND METHODS

Blood donors

Fourteen women (mean age 55, range 35–73) with microscopically confirmed primary mammary carcinoma were examined. Samples of venous blood were obtained six weeks post-operatively, i.e. immediately before the start of radiation therapy, and in connection with the last treatment.

The standard operation was modified radical mastectomy. Nine of the patients had locally advanced disease, i.e. the axillary lymph nodes were involved.

Nine healthy women (mean age 52, range 36–64) were concomitantly examined and served as controls. The same control was used for the first and second examinations.

Radiation therapy

Treatment with [^{60}Co] gamma-irradiation was started six weeks post-operatively. Irradiation was directed to the local lymph node stations and to the chest wall, and the doses were planned individually. A total target dose of 45 Gy (4500 rad) was given over a period of 5 weeks.

Separation of lymphocytes

Heparinized venous blood was centrifuged on Ficoll/Isopaque. Phagocytic cells were removed by iron powder and a magnet, and the lymphocytes were again centrifuged on Ficoll/Isopaque. T cells were separated from

such purified lymphocyte preparations by a rosette sedimentation technique using neuraminidase-treated sheep red cells ($SRBC_n$). The $SRBC_n$–lymphocyte mixture was incubated in 50% fetal calf serum at 4°C overnight. After gradient centrifugation SRBC were lysed with NH_4Cl. The lymphocyte suspension contained 97–100% $SRBC_n$ rosette-forming cells (E_n cells) [1].

Rosette tests

Lymphocytes forming rosettes with $SRBC_n$, considered to represent T cells both with high and low avidity for SRBC, were determined by counting E_n cells before gradient centrifugation (see above). Percentages of total T cells (T_{tot}) were calculated by dividing the number of blood lymphocytes per μl with the percentage of E_n cells. Lymphocytes in T cell-enriched fractions possessing Fc receptors for IgM (T_m cells) or IgG (T_g cells) were identified by their capacity to form rosettes with ox RBC–IgM and IgG complexes respectively. Details of this procedure have been given previously [1]. Duplicates of 400 cells were counted and the results expressed as percentage of total T cells.

Immunofluorescence tests for helper and suppressor T cells

T lymphocyte subsets reacting with fluorescein-isothiocyanate (FITC)-conjugated antibodies against suppressor T cells (LEU 2A) and helper T cells (LEU 3A) (Becton Dickinson Facs Systems, Sunnyvale, CA, U.S.A.) were determined by immunofluorescence. T cells (10^6) were mixed with antibodies in optimal dilutions in phosphate-buffered saline with 5% FCS and incubated for 45 min at 4°C. The cells were washed twice with cold medium and stored on ice before reading with light specific for FITC fluorescence. Alternate illumination with conventional light was applied and 200 cells were counted [3].

Double-labelling experiments

To examine the overlapping between T_g cells and T_s cells, suspensions of T cells from seven healthy donors were first rosetted with ox red cell–IgG complexes, as described above, and then stained with anti-T_s antibodies (LEU 2A), again using the same technique as above. In parallel, the procedure was performed in the reversed order, i.e. T cells were stained with anti-T_s antibodies before T_g rosettes were prepared. The proportions of rosette-forming cells with positive fluorescence and of fluorescence-positive cells forming rosettes were calculated.

Statistical method

Statistical difference between means was calculated using Student's t test.

RESULTS

The absolute numbers of various T lymphocyte subsets are shown in Table 1. All subsets examined decreased by 70–80%. The most pronounced depletion seemed to affect T_g cells.

The proportion of T_g cells was significantly reduced at completion of radiation therapy, whereas T_m cells were significantly increased. In controls the proportions of these T cell subpopulations remained relatively unchanged at the second test. Proportions of T_s and T_h cells were unchanged after radiation therapy and did not change significantly in controls (Table 2).

The proportion of T_g cells in blood was significantly higher in the patient group than in the control group. No other significant differences were noted between values of T cell subsets of patients and controls (Table 2).

Purified T cells were double-labelled with monoclonal antibodies and ox erythrocyte–IgG complexes to elucidate the overlapping between the T_s and T_g subsets. In seven experiments 28 ± 8% of T_g cells reacted with antibodies to T_s cells. With the same T cell suspen-

Table 1. Absolute numbers of lymphocytes and various T lymphocyte subpopulations in blood of breast cancer patients

	No. of tests	Before radiation	After radiation	Relative decrease*
Lymphocytes (μl)	13	1990 ± 190	480 ± 85	76 ± 5
T_{tot} (μl)	9	1720 ± 260	425 ± 95	74 ± 8
T_g (μl)	11	530 ± 90	105 ± 50	81 ± 11
T_m (μl)	6	1070 ± 130	290 ± 80	72 ± 9
T_s (μl)	11	480 ± 120	105 ± 25	77 ± 8
T_h (μl)	7	1010 ± 150	305 ± 90	70 ± 10

Means and 95% confidence limits.
*Percentages of initial values.

Table 2. Percentages of various T lymphocyte subpopulations in blood of patients with breast cancer

	Patients		Controls	
	Before radiation (%)	After radiation (%)	First test (%)	Second test (%)
T_g	(14)*	(11)	(9)	(9)
	28 ± 5†	19 ± 7‡	20 ± 3	21 ± 4
T_m	(13)	(6)	(9)	(9)
	52 ± 7	65 ± 14§	53 ± 8	57 ± 6
T_s	(14)	(11)	(9)	(9)
	25 ± 5	21 ± 4	25 ± 4	23 ± 3
T_h	(14)	(7)	(9)	(9)
	49 ± 5	47 ± 14	52 ± 9	58 ± 7

Means and 95% confidence intervals.
*No. of tests.
†$P < 0.05$ compared with values of controls.
‡$P < 0.02$ compared with values of patients before irradiation.
§$P < 0.05$ compared with values of patients before irradiation.

sions 18 ± 6% of T_s cells formed T_g rosettes. When fluorescence staining was performed before rosetting, the figures were 32 ± 8 and 21 ± 4 respectively.

DISCUSSION

Postoperative radiation therapy has been shown to reduce the development of local recurrence in patients with primary mammary carcinoma [4]. A well-known side effect of this therapy, however, is a profound lymphopenia [5] probably caused by direct damage of lymphocytes. Previous studies have revealed that radiation therapy may cause shifts in T and non-T cell proportions in blood [6, 7]. In the present study we have examined in breast cancer patients the effect of radiation therapy on T cell subpopulations in blood.

Total T cells were reduced to the same extent as total lymphocytes, whereas T_g cells seemed to be somewhat more extensively depleted than were the other subsets examined (Table 1). However, this difference was numerically small and the proportional depletion or enrichment of different suppressor and helper T cell subsets in the purified T cell suspensions obtained after irradiation might better reflect the radiosensitivity of the respective subpopulation.

After radiation therapy a significant reduction in the proportion of blood T_g cells was noted, whereas the proportion of T_m cells was significantly elevated. However, T_s and T_h cell proportions seemed to be unaffected (Table 2). Our earlier results obtained after *in vitro* X-ray exposure of lymphocytes showed T_g cells to be more radiosensitive than T_s cells [3]. This finding might be explained by T_g cells and T_s cells belonging to different populations which only partly overlap. Our present double-labelling experiments showed 20–30% overlapping between T_g and T_s cells (see Results), which supports this interpretation. It has been claimed that 40–80% of T_g cells react with OKT 5 antibodies also defining suppressor/cytotoxic T lymphocytes [8]. It should be mentioned in this context that Reinherz *et al.* reported T_g cells reacting with antibodies against antigens shared with monocytes and granulocytes, and concluded that T_g cells were not true lymphocytes [9]. On the other hand, Fox *et al.* reported that T_g cells bear three out of four T cell-associated antigens [10], and it was shown that T_g cells possess a characteristic pattern of lactate dehydrogenase isoenzymes which is shared by thymocytes and distinct from that of monocytes or myeloid cells [11].

An alternate explanation for the reduction of T cells could be the shedding of Fc receptors for immunoglobulin. This has been described as occurring [12] under particular *in vitro* conditions, and might possibly be increased by X-ray exposure.

In an earlier patient study, proportions of T_g and T_m cells remained essentially constant following pelvic irradiation in patients with urological cancer [13]. A heterogenous clinical status of those patients and differing schedules of radiation therapy might partly explain these diverging results.

In the patient group a higher proportion of T_g cells was noted than in matched controls (Table 2). Most patients with local lymph node involvement had somewhat higher T_g cell proportions than those free of local metastases (data not shown). There was no statistically significant correlation between the clinical variables examined (tumor size and axillary status on histopathological examination) and the proportion of any of the T cell sub-

populations determined. Extended studies would be required to establish such an association. Others have reported raised levels of T_g cells in patients with disseminated cancer [14]. However, in our patients mastectomy had been performed and the tumors were not disseminated. The possibility that the operation by itself might have influenced T_g cell levels by reducing the tumor burden seems less probable in view of the time lapse between surgery and sampling. Moreover, four of these patients were examined half to one year following radiation therapy and then had T_g cell percentages similar to initial values, indicating repopulation of these cells. The relevance of T_g cells for possible host reactions against tumors in man are as yet unclear, but they are known to exert suppression in certain systems [2], and suppressor cells in animals may inhibit effective immune response against tumors [15, 16]. On the other hand, T_g cells may display different cytotoxic functions of possible value for tumor defence, as, for example, antibody-dependent cellular cytotoxicity and 'natural killer' activity [17].

In conclusion, the proportion of T_g cells was higher in operated breast cancer patients than in controls and was reduced following post-operative radiation therapy.

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