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Changes of the Spontaneous Cytotoxicity of the Blood Lymphocyte Population Following Local Radiation Therapy for Breast Cancer

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Abstract—*The capacity of the blood lymphocyte population to spontaneously lyse K562 and Chang cells* in vitro *was examined in women who received local radiation therapy* (45.0 Gy) *for breast cancer. It was observed that cytotoxicity, on a cell-for-cell basis, was significantly reduced against K562 cells at completion of irradiation. This was followed by a recovery to the pretreatment level within* 3–4 months *and remained relatively constant for approximately* 2 yr. *The pattern of these changes were reasonably similar to that of the frequency of lymphocytes expressing Fc-receptors for IgG. In contrast, the relative cytotoxicity against Chang cells was unchanged at completion of irradiation. At* 3–4 months, *however, the relative cytotoxicity was increased above the pretreatment level and remained elevated for at least* 2 yr. *The results indicate that different effector cells are involved in the spontaneous destruction of K562 and Chang cells.*

INTRODUCTION

THE VALUE of adjuvant local radiation therapy in the management of primary breast cancer has been questioned since the treatment may induce a long-lasting lymphopenia which, theoretically, could result in a reduced immunological defence against subclinical disease [1]. Although carefully controlled clinical trials have not demonstrated any enhanced incidence of distant metastases or shorter survival in locally irradiated patients [2, 3], we considered it important to map the radiation-induced changes of the immune system of such patients in more detail.

Previously we have characterized the changes of different subsets of blood lymphocytes after radiation therapy [4–6], and our main effort has been devoted to studies on functional activities of T-cells after irradiation [4, 5, 7–9]. The reason for this is that T-cells were considered to be the most important cell type in the destruction of malignant cells [10]. During recent years, however, lymphocytes capable of spontaneously lysing tumor cells *in vitro* have achieved much attention since animal experiments have indicated a strong correlation between the spontaneous cytotoxic activity of an animal's lymphocytes and its capacity to resist tumor transplants [11–14]. Although the role of such a first-line defence in the control of human tumors is far from established, we considered it of importance to examine how radiation therapy affects the capacity of the blood lymphocyte population to spontaneously kill tumor cells *in vitro*. An extension of our previous work on this subject [15] is now presented.

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MATERIALS AND METHODS

Patients and controls

Forty-five women with primary breast cancer were studied. Their ages ranged from 28 to 67 years, with a mean of 52. One of the patients received local radiation therapy (see below) for a local recurrence. The others had recently been operated on for breast cancer with histopathologically verified tumor involvement of axillary nodes and/or primary tumors exceeding 3 cm in diameter. During the course of this investigation distant metastases were detected in seven of the patients, a local recurrence in one, and in one patient a new cancer developed in the remaining breast.

Twenty-three healthy women, 22–63 years of age, with a mean of 49, served as controls.

Treatment of the patients

The patients underwent a modified radical

mastectomy followed by local radiation therapy 4–6 weeks later. In one patient, however, radiation therapy was given for a local recurrence 2 years after primary surgery. All the patients received radiation therapy, using 6–8 MeV electrons and a [^{60}Co] source, to the chest wall, internal mammary region, the supraclavicular fossa and axilla, as described [3]. A total target dose of 45.0 Gy (4500 rad) was delivered to all regions in approximately 5 weeks.

The postmenopausal women of this investigation were included in a randomized trial aimed at establishing the clinical value of adjuvant antiestrogen (tamoxifen, ICI) treatment. Nine out of 23 postmenopausal women received this drug, at a daily oral dose of 40 mg. This treatment started at the beginning of the radiation therapy and continued throughout this study.

Blood sampling

Venous blood was obtained from the patients on 8 occasions. Sample I was obtained within 1 week before postoperative radiation was started. The subsequent samples were obtained at the following time periods after completion of irradiation: sample II within 1 week, sample III at 3–4 months, sample IV at 6–8 months, sample V at 10–12 months, sample VI at 14–16 months, sample VII at 12–23 months and sample VIII at 25–29 months.

Blood sampling was stopped when a patient developed recurrent disease or a new cancer. Frequently, samples were not obtained for trivial reasons.

Separation of lymphoid cells

Lymphoid cells were separated from heparinized blood by centrifugation on Ficoll–Isopaque and phagocytic cells were removed magnetically [16].

Cytotoxic test

The method used for measuring the spontaneous cytotoxicity of lymphocytes has been described [17]. In short, various numbers of lymphocytes were incubated with 10^4 [^{51}Cr]-labelled Chang cells (derived from human liver) or K562 cells (derived from a human myeloid leukemia) for 4 hr at 37°C. The amount of released isotope was then measured and a cytotoxic index was calculated according to the following formula:

$$\frac{\%\text{ release with lymphocytes} - \%\text{ spontaneous release}}{100 - \%\text{ spontaneous release}}.$$

All cultures were set up in duplicate. Variability within the duplicates usually did not exceed 10%.

Rosette test

Lymphocytes expressing membrane-bound Fc-receptors for IgG (FcR) were detected by a rosette technique using ox erythrocytes sensitized with a rabbit anti-ox erythrocyte IgG. Lymphocytes binding 3 or more such complexes will be termed EA rosette-forming. The technique has been described [18].

Data processing and statistical evaluation

Results which were obtained within 3 months of detection of recurrent disease or a second malignancy are not included in the calculations. The initial value (sample I) for cytotoxic activity and lymphocyte number of each patient was put as 100% and the subsequent values of the same patient related to this. Student's *t*-test was used to calculate whether there were statistically significant differences between groups of observations.

RESULTS

Blood lymphocyte counts and frequency of EA rosette-forming lymphocytes after irradiation

The number of lymphocytes per μl of blood was counted in most of the patients before and at various times after radiation therapy. Determinations of the frequency of EA rosette-forming lymphocytes was performed in 17 of these patients. In 6 cases determinations were not performed before and at completion of radiation therapy (samples I and II).

Figure 1 shows that the lymphocyte counts were reduced to approximately 30% at completion of irradiation, followed by an increase up to approximately 60% 25–29 months after treatment. The proportion of EA rosette-forming lymphocytes was reduced from approximately 20 to 10% at termination of radiation therapy, followed by a restoration up to or possibly above the pretreatment level.

Spontaneous cytotoxicity of blood lymphocytes after irradiation

Cytotoxicity of lymphocytes for K562 (Fig. 2) and Chang (Fig. 3) cells was tested before and after radiation therapy at lymphocyte:target

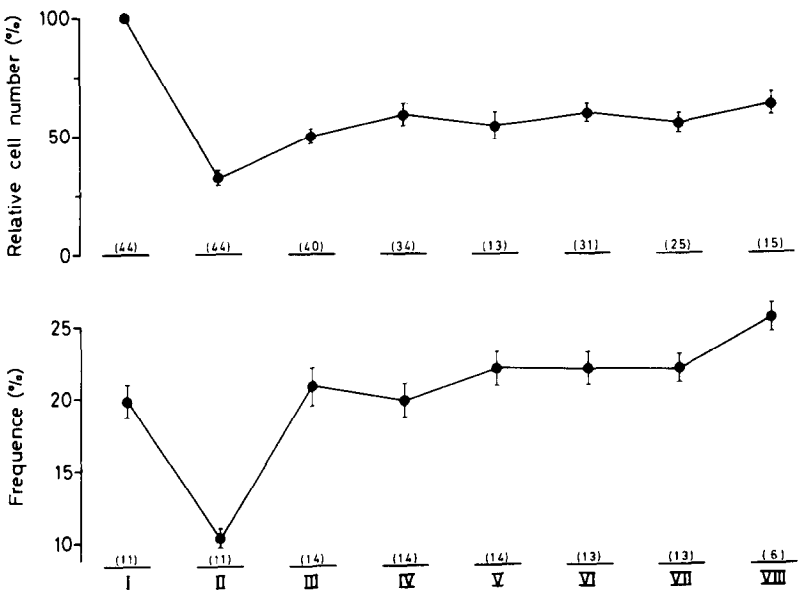

Fig. 1. Relative changes of lymphocyte counts and frequency of EA rosette-forming lymphocytes following radiation therapy. The number of patients tested are shown within parentheses and symbols represent mean values ± S.E. The time periods for blood sampling are described in Materials and Methods. Following irradiation (samples II–VIII) the lymphocyte counts were significantly reduced ($P < 0.001$). *The frequency of EA rosette-forming cells was only significantly changed in sample II* ($P < 0.005$). *The mean absolute lymphocyte counts ± S.E. in sample I were* 2200 ± 120 *per* μl *of blood.*

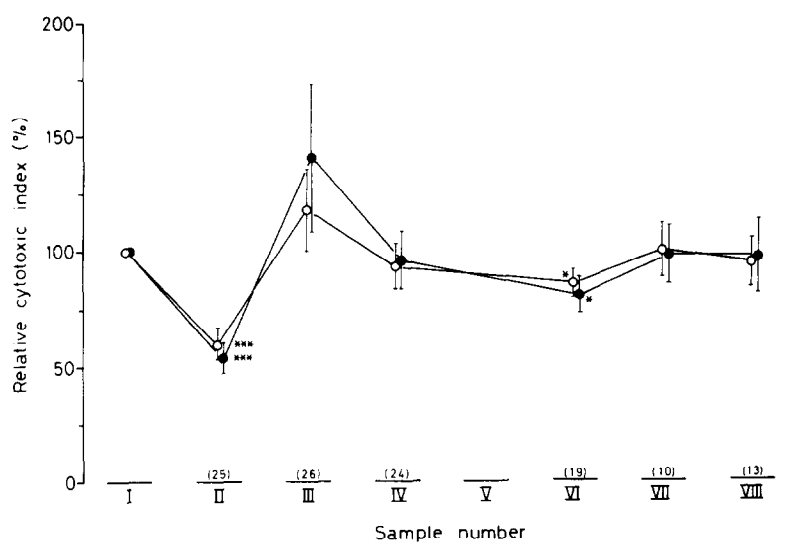

Fig. 2. Relative spontaneous cytotoxicity of blood lymphocytes for K562 cells following radiation therapy. Lymphocyte: target cell ratio of 100:1 (○———○) *and* 50:1 (●———●). *The number of patients tested are shown within parentheses and symbols represent mean values ± S.E. Asterisks at the symbols indicate that the changes were statistically significant.* * * * = $P < 0.001$, * = $P < 0.05$.

cell ratios of 25:1, 50:1 and 100:1. The results using the lowest ratio are not presented since they were extremely variable.

The absolute values of the cytotoxic indices of the patients before treatment (sample I) and healthy controls are presented in Table 1. It can be seen that K562 cells were more susceptible than Chang cells and there were no significant differences between patients and controls.

The relative cytotoxicity against K562 cells was reduced to approximately 60% at completion of irradiation, followed by a restoration 4–5 months later. During the rest of the obser-

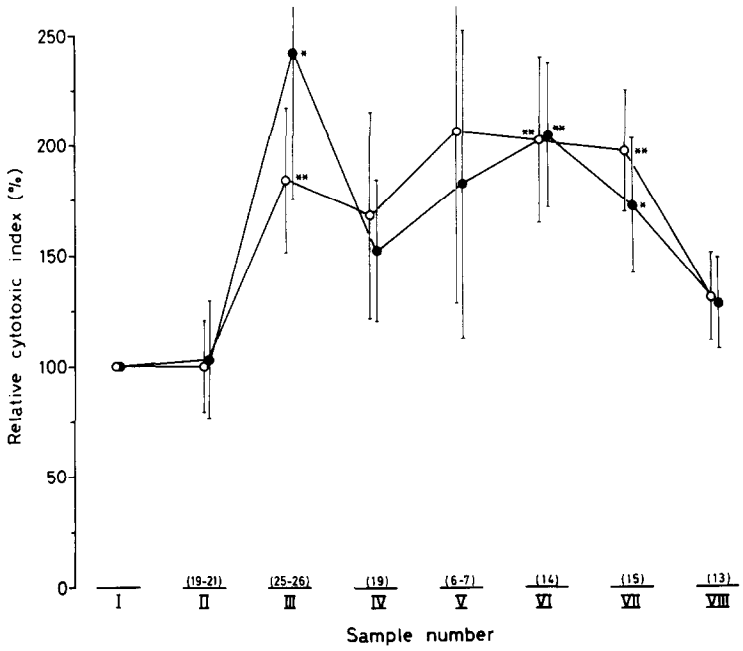

Fig. 3. Relative spontaneous cytotoxicity of blood lymphocytes for Chang cells following radiation therapy. Lymphocyte: target cell ratio of 100:1 (○———○) *and* 50:1 (●———●). *The number of patients are shown within parentheses. Means ± S.E. are presented. Asterisks at the symbols indicate that the changes were statistically significant.* * * * = $P < 0.001$, * * = $P < 0.01$, * = $P < 0.05$.

Table 1. Spontaneous cytotoxicity of lymphocytes obtained from patients before radiation therapy (sample 1) and healthy controls against K562 and Chang cells

	K562 cells		Chang cells	
	50:1	100:1	50:1	100:1
Patients	0.51 ± 0.03 (37)	0.59 ± 0.03 (37)	0.15 ± 0.02 (38)	0.21 ± 0.03 (38)
Controls	0.39 ± 0.05 (17)	0.53 ± 0.05 (17)	0.12 ± 0.01 (23)	0.20 ± 0.02 (23)

Mean cytotoxic indices ± S.E. are shown using two different lymphocyte:target cell ratios. No significant differences were observed between patients and controls. Numbers within parentheses indicate the number of individuals tested.

vation period of 2 years the relative cytotoxicity remained at or slightly below the pretreatment level.

The relative cytotoxicity against Chang cells was not changed at completion of irradiation. Notwithstanding the extensive variability, cytotoxicity seemed to increase above the pretreatment level 3–4 months after irradiation and seemed to remain elevated for almost 2 years. Some reduction possibly occurred 25–29 months after irradiation.

To exclude the possibility that the above results can be explained by a changed sensitivity of the assay with time, the sensitivity of the target cells were compared during different years of this investigation (Table 2). Although not statistically significant, the results suggest that the sensitivity of both K562 and Chang cells had decreased somewhat with time.

DISCUSSION

The impact of local radiation therapy for breast cancer on the blood lymphocyte population has been studied by several groups. This treatment has been shown to induce a long-lasting reduction of the number of circulating T lymphocytes, whereas the non-T lymphocyte population recovers more rapidly. In addition, the proliferative responses of the cells to non-

Table 2. Sensitivities of K562 and Chang cells to spontaneous cytotoxic lymphocytes during the first 3 years of the study

Year of test	Number of patients	Chang cells			K562 cells		
		25:1	50:1	100:1	25:1	50:1	100:1
1978	17	0.13 ± 0.04	0.19 ± 0.05	0.27 ± 0.06	0.41 ± 0.05	0.54 ± 0.04	0.65 ± 0.04
1979	15	0.08 ± 0.02	0.15 ± 0.02	0.20 ± 0.05	0.40 ± 0.05	0.52 ± 0.05	0.58 ± 0.05
1980	10	0.09 ± 0.02	0.13 ± 0.03	0.20 ± 0.03	0.30 ± 0.06	0.43 ± 0.07	0.53 ± 0.06

The lymphocytes were obtained from patients before radiation therapy (sample I). Mean cytotoxic indices ± S.E. using 3 different lymphocyte:target cell ratios are presented. No significant time-dependent alterations of the sensitivities were observed.

specific and specific mitogenic stimuli are reduced, indicating impaired T cell functions after irradiation (for a review see [19]). The lack of correlation between the occurrence of recurrent disease and the extent of radiation-induced depression and subsequent recovery pattern of the mitogenic responses of lymphocytes to PPD [20, 21] stimulated us to examine other immunological functions which have also been implicated in the defence of malignant cells.

In this investigation we have examined the capacity of the blood lymphocyte population to spontaneously kill K562 and Chang cells *in vitro*. These lytic activities are probably mainly mediated by natural killer (NK) cells. The extent by which other cells such as so-called K cells, mediating antibody-dependent cellular cytotoxicity (ADCC), contribute to the killing is not known.

The ideal way of presenting the results would be to express cytotoxicity in lytic units per volume of blood. However this was not possible since in some tests the slopes of the dose–response curves, using increasing lymphocyte:target cell ratios, were close to zero. Therefore, the results are expressed as cytotoxicity per unit cell number. It was observed that the relative capacity of lymphocytes to lyse K562 cells were significantly reduced at completion of radiation therapy, followed by a recovery within 3 or 4 months (Fig. 2). The pattern by which the relative lytic activity changed was reasonably similar to that of the frequency of EA rosette-forming cells (Fig. 1). This indicates that the alterations of cytotoxicity against this target cell were due to shifts in the frequency of lymphocytes with FcR known to be present on K cells and most NK cells (for a review see [22]). Our results on the changes of cytotoxic activity against K562 cells seem to be concordant with those of O'Toole *et al.*, who showed that radiation therapy for carcinoma of the urinary bladder reduced the cytotoxic activity of blood lymphocytes against allogeneic bladder cancer cells [23]. Moreover, the K cell activity in the blood was observed to be reduced shortly after completion of radiation therapy for various types of malignancies [24–26].

The relative cytotoxic activity for Chang cells appeared to change in a pattern quite different from that of K562 cells (Fig. 3). The activity was unchanged at completion of irradiation, followed by an increase above the pretreatment level, and the activity remained elevated for at least 2 years. Thus, the pattern of these changes did not bear any similarity to that of FcR-positive lymphocytes, indicating that there is no simple relation between the extent of killing and the proportion of FcR-bearing cells. Our results on the cytotoxicity against Chang cells are similar to those of other investigators using other target cells. For instance, cytotoxicity of blood lymphocytes against allogeneic lung cancer cells was observed to be increased at completion of irradiation for this disease [27, 28]. A recent report showed that the spontaneous cytotoxicity of blood lymphocytes against K562 targets is significantly increased 3–5 years after radiation therapy for endometrial cancer [29]. Others observed that spontaneous cytotoxicity of blood lymphocytes against allogeneic ovarian carcinoma targets was reduced during the first weeks of irradiation, followed by a recovery which started during continuation of the treatment [30]. Thus most workers have observed that local radiation therapy augments the spontaneous cytotoxicity of the blood lymphocyte population for allogeneic tumor cells. This may be due to a selective survival after irradiation of relevant cytotoxic cells. This thesis is supported by the finding that the spontaneous cytotoxicity of human blood is relatively radioresistant, having a D_0-value of 750–850 rad. In addition, low

doses of irradiation may augment the cytotoxic activity of lymphocyte preparations [31, 32]. Another possibility could be that radiation therapy depletes cells which suppress the function of spontaneously cytotoxic cells. Such cells have been described [33]. A third possibility could be that the spontaneous cytotoxicity was suppressed in the patients before the treatment and that it became normalized after treatment. This explanation, however, seems unlikely since the cytotoxic activities of their lymphocytes were similar to those of healthy controls (Table 1). A fourth possibility could be that the tamoxifen treatment of the patients had increased the spontaneous cytotoxicity. However, because of the relatively high variability of the determinations and the low numbers of patients treated with this drug, we cannot draw any such conclusion (data not shown).

In summary, our results have shown that the effects of local radiation therapy on the spontaneous cytotoxicity of blood lymphocytes may differ depending on the target cells used. This indicates that different effector cells may be involved in the killing of different target cells.

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