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NATURAL KILLER ACTIVITY IN PERIPHERAL LYMPHOCYTE POPULATION FOLLOWING LOCAL RADIATION THERAPY

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Lymphocytes which express natural cytotoxicity for tumour cells in vitro have recently received much interest because of their possible role in surveillance mechanisms. A positive correlation has been found in experimental animals between natural killer (NK) activity in vitro and capacity to prevent growth of transplanted tumours (HALLER et coll. 1977, KASAI et coll. 1979, KIESSLING et coll. 1975, WARNER et coll. 1977). Moreover, mouse strains displaying a high incidence of spontaneous leukemia exhibit low NK activity in vitro and vice versa (ZARLING et coll. 1975).

Although the role of NK cells in tumour cell surveillance in the human is unknown, it is of interest to examine whether they are affected by treatments which are frequently used to control human malignant tumours. In the present investigation the NK activity in peripheral lymphocytes has been examined after postoperative radiation therapy for breast carcinoma. Previously it has been shown that this treatment induces lymphopenia with an alteration in the proportion of lymphocyte subpopulations (PETRINI et coll. 1977), reduces the responses of the lymphocytes to soluble antigen and allogeneic cells (BARAL et coll. 1977, BLOMGREN et coll. 1977) and reduces the capacity of the cell population to mediate antibody dependent cellular cytotoxicity (ADCC; WASSERMAN et coll. 1975).

Material and Methods

The material consisted of 24 women with primary breast carcinoma (32–70 years old), 6 patients with carcinoma of the prostate (62–77 years old) and 7 patients with carcinoma of the urinary bladder (1 female and 6 males with an age range of 61–77 years). The patients with breast carcinoma were considered operable at diagnosis but not the patients with carcinoma of the prostate or of the bladder.

Irradiation techniques. Patients with breast carcinoma were treated with a modified radical mastectomy followed by local irradiation up to a total target dose of 45.0 Gy (4 500 rad) as described by IDESTRÖM et coll. (1979). Patients with carcinoma of the prostate received local irradiation of the tumour covering the entire small pelvic region but not extending to the juxta-regional nodes. The calculated mean tumour dose of 54.0 Gy was given in 6 weeks. Patients with carcinoma of the bladder received irradiation of the bladder with a wide margin including the regional lymph nodes in the 75 per cent isodose curve. The calculated total tumour dose was 64.5 Gy given during 8 weeks with a break of 2 weeks after 32.0 Gy. Patients with carcinoma of the prostate or

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bladder were treated with 6 MV roentgen rays using a 3-field technique with 2 wedge filter beams in the front and one open beam from the back.

Blood sampling. Blood samples were obtained from the patients with breast carcinoma at four occasions: sample number I within one week before postoperative irradiation was started, sample II within one week after completion of irradiation, sample III three to four months after irradiation and sample IV six to eight months after irradiation. Two blood samples were obtained from the patients with carcinoma of the urinary bladder or the prostate: within one week before start of irradiation and within one week after a total target dose of 54.0 Gy (carcinoma of the prostate) and 64.5 Gy (carcinoma of the urinary bladder).

Separation of lymphocytes. Lymphoid cells were separated from heparinized venous blood by centrifugation on Ficoll-Isopaque and phagocytic cells were removed magnetically (BLOMGREN 1974). The resultant preparations were used for testing the NK activity of the lymphocyte preparation. T-cells were separated from these purified lymphocyte preparations by rosetting with neuraminidase treated sheep red blood cells (E_N) followed by centrifugation on Ficoll-Isopaque (MORETTA et coll. 1977).

Rosette techniques. Details of these techniques have been presented previously (PETRINI et coll. 1979). Lymphocytes possessing membrane receptors for the Fc-part of IgG were identified in non-separated preparations by their capacity to form rosettes with ox RBC sensitized with rabbit anti ox RBC IgG. Lymphocytes in T-cell enriched fractions possessing Fc-receptors for IgG (T_G-cells) or IgM (T_M-cells) were identified by their capacity to form rosettes with ox RBC IgG and IgM, respectively. The frequency of T-cells was established by counting cells forming rosettes with E_N.

Cytotoxic test. NK activity was measured by incubating lymphocytes for 4 h with ^{51}Cr-labelled target cells termed K562 (derived from a human myeloid leukemia) and Chang cells (probably derived from human liver) as described previously (EINHORN et coll. 1978). A cytotoxic index was calculated according to the following formula: (% release with lymphocytes–% spontaneous release)/(100–% spontaneous release).

Duplicate tests were performed and variability within the duplicates did not exceed 10 per cent.

Statistics. Values obtained after radiation therapy were related to the pretreatment value of each patient. The Student's t-test was used for calculating whether the changes were statistically significant.

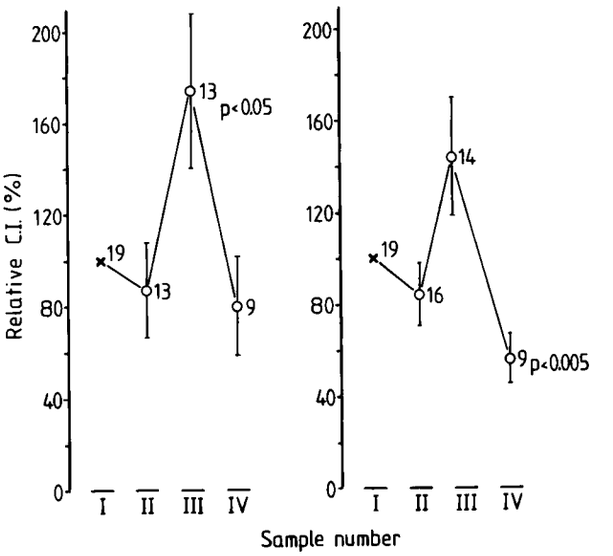

Fig. 1. Relative changes of the NK activity, expressed as a cytotoxic index (C.I.), of the peripheral lymphocyte population against Chang cells after irradiation for mammary carcinoma. Left diagram shows the results employing a lymphocyte: target cell ratio of 50 : 1 and the right diagram 100 : 1. Pretreatment values are set as 100 per cent. The absolute pretreatment cytotoxic indices (mean ± SE) were 0.16±0.04 and 0.22±0.04, respectively. Mean values ± SE are shown and figures at the symbols indicate the number of patients. A p-value at a symbol indicates that this mean differs significantly from the pretreatment value.

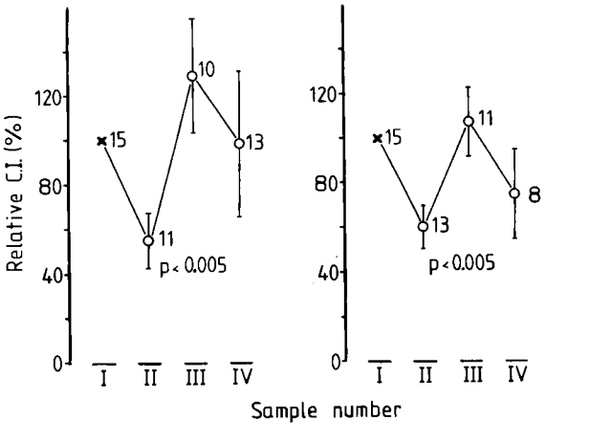

Fig. 2. Same as in Fig. 1 except that K562 cells were used as target cells. The absolute pretreatment cytotoxic indices (mean ± SE) were 0.54±0.05 using a lymphocyte : target cell ratio of 50 : 1 and 0.63±0.04 using a ratio of 100 : 1.

Results

NK activity of peripheral lymphocyte population following irradiation of breast carcinoma. The NK

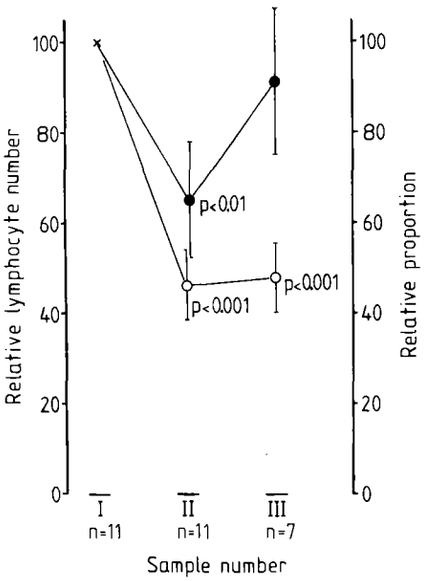

Fig. 3. Relative lymphocyte counts and relative proportions of lymphocytes possessing Fc-IgG receptors in non-fractionated cell preparations after irradiation of mammary carcinoma. The pretreatment values are set as 100 per cent. The absolute pretreatment lymphocyte count (mean ± SE) was 2000±200 and the percentage of Fc-IgG bearing cells 19.7±2.4. Mean values ± SE are shown. A p-value at a symbol indicates that this mean value differs significantly from the pretreatment value. Relative lymphocyte counts (○), relative proportion of lymphocytes possessing Fc-IgG receptors (●).

Table

Percentage of lymphocytes forming rosettes with E_N in non-fractionated lymphocyte preparations and the frequency of lymphocytes possessing Fc-receptors for IgG (T_G) and IgM (T_M) in E_N rosette sedimented fractions. The determinations were made in 13 patients before irradiation and at completion of pelvic irradiation. Mean values ± SE are shown

	Before irradiation	After irradiation
E_N binding cells	79.9±2.0	73.2± 3.5 (92.2± 4.8)*
T_G-cells	22.5±1.6	24.6± 5.0 (115.0±23.1)
T_M-cells	37.3±3.5	39.9± 6.6 (110.8±16.0)

* Mean values ± SE expressed as per cent of the pretreatment values. No statistically significant changes after irradiation.

activity of the lymphocyte population exhibited a small but non-significant reduction for Chang cells at completion of radiation therapy for breast carcinoma (Fig. 1). This was followed by a significant overshoot three to four months later employing a lymphocyte-target cell ratio of 50: 1. Six to eight months after irradiation (sample 4), NK activity was reduced to the original level or below this level. Most of the patients in this group were also examined for NK activity against K562 cells (Fig. 2). The NK activity against this target cell largely changed similar to that recorded against Chang cells with the exception that the initial reduction was statistically significant and the ensuing increase less marked.

Frequency of Fc-receptor bearing lymphocytes following irradiation. Since lymphocytes mediating NK activity in the human seem to possess Fc-receptors for IgG (COOPER et coll. 1977), the proportion of such cells following irradiation was examined. The changes of the lymphocyte counts and the relative proportion of lymphocytes possessing Fc-receptors for IgG following irradiation for breast carcinoma appear in Fig. 3. Five of the patients included in this group were also tested for NK activity. The lymphocyte counts were reduced to approximately 45 per cent and the relative proportion of Fc-IgG positive cells was reduced to approximately 65 per cent at completion of irradiation. Three to four months after treatment (sample 3) the lymphocyte counts remained at essentially the same level, whereas the frequency of Fc-IgG positive cells had recovered to approximately 90 per cent of the original value.

Since the NK cell is considered to be a non-T-cell by COOPER et coll. and since the T-cell population in the blood also contains Fc-IgG receptor positive cells (T_G), it was of interest to know whether the decline of the proportion of Fc-receptor bearing cells was due to a reduced frequency within the T or the non-T-lymphocyte compartments. This was analysed in a group of patients who received radiation therapy to the pelvic region and whose lymphocyte counts were reduced to 40 to 60 per cent of the pretreatment values. The frequency of lymphocytes forming E_N rosettes did not change (Table) and the frequency of T_G cells within this cell population was not altered significantly. The proportion of T_M cells also remained essentially constant.

Discussion

The NK activity of the peripheral lymphocyte population was analysed on a cell-for-cell basis, before and after postoperative radiation therapy for breast carcinoma. Such knowledge may be relevant since data from animal experiments indicate a close

correlation between in vitro NK activity and growth of tumour cells in vivo.

Somewhat different results were obtained depending on the type of target cell and the lymphocyte target cell ratio. NK activity against K562 cells was significantly reduced shortly after completion of irradiation whereas such a reduction was hardly discernible against Chang cells (Figs 1, 2). Three to four months after irradiation the NK activity against Chang cells was above the pretreatment level followed by a decline. No significant overshoot was observed using K562 cells as targets. These results may indicate that the lymphocyte population mediating NK activity is heterogeneous with respect to radiation sensitivity and mode of recovery after irradiation. Moreover, as shown in Figs 1 and 2, the amplitudes of the changes of the NK activity after irradiation may be dependent on the lymphocyte target cell ratio.

The frequency of lymphocytes possessing Fc-receptors for IgG in non-fractionated lymphocyte preparations varied similarly to that of the NK activity against K562 cells (Fig. 3). These lymphocytes may be mainly non-T-cells as the frequency of Fc-IgG receptor bearing lymphocytes within the T-cell population was not significantly changed in patients irradiated for pelvic tumours (Table). Provided that these data can be applied to patients with breast carcinoma, it is possible that the fluctuations of the relative NK activity may at least partly be due to a changed frequency of Fc-IgG bearing non-T-cells.

It has been observed that local radiation therapy for pulmonary carcinoma augments the cytotoxicity of the peripheral lymphocytes against allogeneic pulmonary carcinoma cells as measured one week after completion of therapy (MANABE et coll. 1977). Radiation therapy for ovarian carcinoma stage III did not change the cytotoxicity of the peripheral lymphocytes against allogeneic ovarian carcinoma cells in spite of a profound radiation-induced lymphopenia. In contrast, radiation therapy for ovarian carcinoma stage I and II caused a significant reduction of cytotoxicity after seven days of treatment which, however, recovered during continuation of treatment (KOHORN et coll. 1978). A reduction of cytotoxicity of peripheral lymphocytes against allogeneic urinary bladder carcinoma cells after irradiation was observed by O'TOOLE et coll. (1973).

It should be emphasized that, in the investigations mentioned, the target cells for cytotoxicity were of the same histologic type as the patient tumours and probably partially of ADCC type. Therefore, these results may not be strictly comparable with those of the present report. However, several experiments performed in animals have shown that the NK cell is relatively resistant to radiation as measured within 24 h of irradiation (HOCHMAN & CUDKOWICS 1977, KIESSLING et coll. 1977, OEHLER & HERBERMAN 1978). HOCHMAN et coll. (1978) observed that the NK activity in the spleen did not decline until 12 days after whole body exposure of mice to about 7 Gy (originally 700 R) and recovery started on day 28. They concluded that mature NK cells are relatively radiation resistant whereas their progenitors are relatively sensitive. In contrast, it was concluded by OEHLER & HERBERMAN that rat pre-NK cells are more resistant than mature NK cells, since exposure of rats to about 10 Gy (originally 1 000 R) abolished the NK cell activity which, however, was restored by treating animals with poly I: C, a possible activator of pre-NK cells.

The fluctuations of the NK activity after radiation therapy as observed in the present investigation may indicate that mature NK cells or their progenitors, or both, are affected by the treatment. In an attempt to elucidate this question an investigation was started to reveal whether these fluctuations disappear by treating the lymphocytes with interferon which is known to augment NK activity (EINHORN et coll., TRINCHIERI & SANTOLI 1978). Additional tests may also reveal whether the NK activity of the patient lymphocytes correlate to prognosis of the disease.

SUMMARY

The natural killer (NK) activity of peripheral lymphocytes against ^{51}Cr-labelled Chang or K562 cells was tested using a 4 h release assay before and after postoperative radiation therapy for mammary carcinoma. NK activity against K562 was significantly reduced at completion of therapy (a total target dose of 45.0 Gy) and restituted 3 to 4 months later. NK activity against Chang cells exhibited a slight but non-significant decline at completion of therapy followed by an overshoot 3 to 4 months later. The frequency of Fc-IgG receptor bearing lymphocytes was decreased at completion of therapy and largely restored after 3 to 4 months.

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