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SPONTANEOUS CYTOTOXICITY OF BLOOD LYMPHOCYTES FOLLOWING LOCAL RADIATION THERAPY FOR CARCINOMA OF THE URINARY BLADDER

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There is a general agreement that local radiation therapy may cause a long lasting lymphopenia. However, there are considerable controversies concerning the effects of such treatment on the size of various subsets of blood lymphocytes and their functions. These divergent results may be due to different techniques for separating the lymphocytes, different assays for measuring the same biologic activity and the timing of the tests in relation to the therapy. Another possibility could be that radiation therapy directed to different regions of the body produces different effects on the blood lymphocyte population.

In a previous investigation, BLOMGREN et coll. (1982a) observed that the results on the spontaneous cytotoxicity of blood lymphocytes after radiation therapy for breast carcinoma seemed to differ from those obtained in patients irradiated for endometrial carcinoma (ONSRUD & THORSBY 1981). Data on the effects of local radiation therapy for bladder carcinoma on the spontaneous cytotoxicity of blood lymphocytes are now presented. The results obtained were in agreement with those of ONSRUD & THORSBY supporting the view that irradiation of different parts of the body may change immunologic reactivities of blood lymphocytes in different directions.

Material and Methods

The material comprised 45 patients (9 females, 36 males), aged 55 to 80 years (mean 70), with a histopathologically confirmed diagnosis of transitional cell carcinoma of the urinary bladder. According to UICC classification (Union Internationale Contre le Cancer 1974) the tumor stages were T2, T3 or T4. None of the patients had undergone any major surgery for their tumor or previously received any treatments with ionizing irradiation or cytotoxic drugs.

Radiation therapy. All patients received a full dose of radiation therapy directed to the bladder with a wide margin including the regional lymph nodes in the 75 per cent isodose using 6 MV roentgen rays delivered by a linear accelerator. A three-field technique was employed with two wedge filter beams in the front and one open beam from the back. A total target dose of 64 Gy was delivered during 8 weeks with a break of 2 weeks after 32 Gy.

Blood sampling. A group of 29 patients was tested serially. The first blood sample was obtained within 5 weeks before radiation therapy was started and the second sample was drawn on the same day that radiation therapy was terminated. Sample III was obtained 4 to 6 weeks after irradiation. The subsequent samples were obtained at the time periods indicated in the figures with an accuracy of approximately ±2 weeks except for the last sample which was drawn 16 to 19 months after irradiation.

Blood sampling was discontinued in 10 patients because of progressive deterioration or due to the

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fact that they were entered on treatments with cytotoxic drugs. In addition, some blood samples were not drawn for more trivial reasons.

Separation of lymphocytes. Lymphoid cells were separated from heparinized venous blood by centrifugation on Ficoll-Isopaque and phagocytic cells were removed magnetically after addition of carbonyl iron powder as described previously (BLOMGREN 1974). More than 98 per cent of the cells were classified as lymphocytes and viability of the cells, as assessed by Trypan blue staining, always exceeded 95 per cent.

Identification of lymphocytes with Fc-IgG receptors. Lymphocytes with membrane associated receptors for the Fc-part of IgG (FcR) were identified by their capacity to form rosettes with ox-erythrocytes sensitized with rabbit anti-ox-erythrocyte IgG as described before (PETRINI et coll. 1979). Cells forming rosettes with such complexes will be termed EA-rosette forming.

Spontaneous cytotoxicity of lymphocytes. This technique has been described previously (EINHORN et coll. 1978). In short, varying numbers of lymphocytes were incubated with 10^4 ^{51}Cr-labelled allogeneic target cells termed Chang and K562. The former are derived from human liver and the latter from a human myeloid leukemia. The lymphocyte: target cell ratios were set at 50 : 1, 25 : 1 and 12.5 : 1.

All tests were set up in duplicate. After 4 h of incubation at 37°C the proportion of released radiation activity was determined and a cytotoxic index was calculated according to the following formula: (% release with lymphocytes−% spontaneous release)/(100−% spontaneous release).

Presentation of data. All values concerning a patient were expressed as percentage of the initial value in this patient. The geometric mean values were used. Statistical significance between groups of observations was estimated using the Student's t-test.

Results

Blood lymphocyte population after pelvic irradiation

Changes of lymphocyte counts and proportion of EA-rosette forming cells. Fig. 1 shows the changes of the lymphocyte counts in the blood and the relative frequency of EA-rosette forming cells various times after local radiation therapy for bladder carcinoma. The absolute pretreatment lymphocyte count per µl of blood was 1740±120 and the absolute

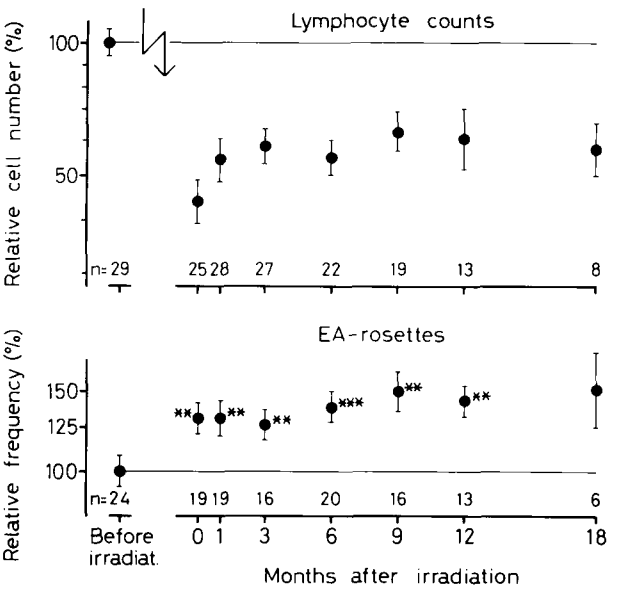

Fig. 1. Changes of lymphocyte counts in the blood and the relative frequency of EA-rosette forming lymphocytes following local radiation therapy for breast carcinoma. Mean values ± SE. The number of patients tested is indicated at the abscissa. Asterisks at the symbols indicate that the changes are statistically significant. **=p<0.01 and ***=p<0.001.

Table 1

Relative lymphocyte counts and frequency of EA-rosette forming lymphocytes in the blood of a group of 16 patients at completion of pelvic irradiation (64 Gy). The results of these patients are also pooled with those presented in Fig. 1. Mean log_{10} per cent values ± SE are presented. Values within parentheses show the antilogarithmized mean values

Before irradiation		At completion of irradiation	
Lymphocyte counts (n=16)	2.000 (100%)*	1.600±0.040 (39.8%)	p<0.001
Proportion of EA-rosettes (n=16)	2.000 (100%)**	2.071±0.035 (117.8%)	NS
Proportion of EA-rosettes, pooled data (n=35)	2.000 (100%)***	2.096±0.023 (124.7%)	p<0.001

* Absolute pretreatment lymphocyte count/µl of blood was 1870±149.

** Absolute percentage of EA-rosette forming lymphocytes was 24.3±3.2.

*** Absolute percentage of EA-rosette forming lymphocytes was 22.3±1.8.

percentage of EA-rosette forming cells was 20.1±1.7 (M ± SE).

At completion of irradiation the lymphocyte counts were reduced to approximately 45 per cent of

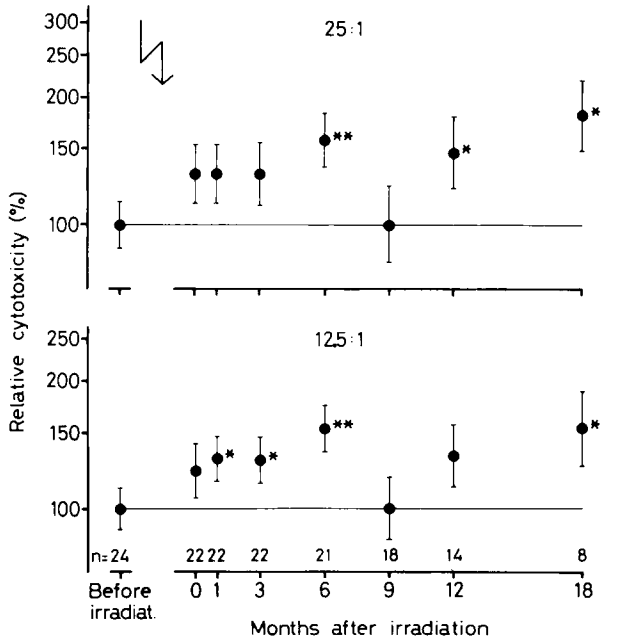

Fig. 2. Relative spontaneous cytotoxicity of blood lymphocytes against K562 cells various times after local radiation therapy for bladder carcinoma. Cytotoxicity was tested at lymphocyte : target cell ratios of 25 : 1 and 12.5 : 1. Mean values ± SE. Asterisks at the symbols indicate that the changes are statistically significant. *=p<0.05 and **=p<0.01.

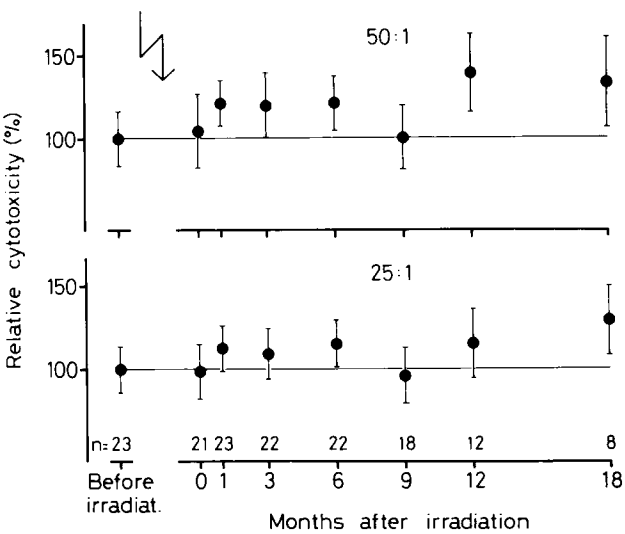

Fig. 3. Relative spontaneous cytotoxicity of blood lymphocytes against Chang cells various times after local radiation therapy for bladder carcinoma. Cytotoxicity was tested at lymphocyte : target cell ratios of 50 : 1 and 25 : 1. Mean values ± SE.

the initial value followed by a slight but statistically non-significant recovery during the subsequent 18 months. At all time periods the lymphocyte counts were significantly lower than the pre-treatment values (p<0.01 or p<0.001; data not presented). The relative frequency of EA-rosette forming lymphocytes was significantly increased (130 per cent) at completion of irradiation and remained increased during the rest of the observation period.

Since the frequency of EA-rosette forming lymphocytes is sharply reduced at completion of local radiation therapy for breast carcinoma (BLOMGREN et coll. 1982a) another group of 16 patients with bladder carcinoma was analyzed before and at completion of irradiation (Table 1). Although not statistically significant the proportion of EA-rosette forming cells also increased in these patients. When the results of these patients were pooled with those presented in Fig. 1 the increase reached a highly significant level (p<0.001).

Changes of spontaneous cytotoxicity. The relative spontaneous cytotoxicity of blood lymphocytes for Chang cells, K562 cells or both was examined in the patients presented in Fig. 1. Cytotoxicity against Chang cells was generally very low or not measurable using an effector : target cell ratio of 12.5 : 1. These results are therefore not presented. Cytotoxicity against K562 cells was clearly measurable in all tests at all effector : target cell ratios. However, in several patients there was no further increase of cytotoxicity by increasing the ratio from 25 : 1 to 50 : 1. For this reason the results of the latter ratio are not presented.

Figs 2 and 3 show that cytotoxicity tended to increase against both target cells after radiation therapy. This increase was most marked against K562 targets and reached statistical significance at several time points.

Since there is a significant reduction of the relative spontaneous cytotoxicity against K562 cells at completion of radiation therapy for breast carcinoma (BLOMGREN et coll. 1982a) another group of patients, tested for spontaneous cytotoxicity after pelvic irradiation, was analyzed (Table 2). In agreement with the results presented in Figs 2 and 3 there was no tendency for radiation therapy to reduce the relative cytotoxicity. In contrast cytotoxicity seemed to increase.

Discussion

In a previous investigation the spontaneous cytotoxicity of the blood lymphocyte population following radiation therapy for breast carcinoma was examined. The relative cytotoxicity against Chang

Table 2

Relative spontaneous cytotoxicity against K562 and Chang cells of blood lymphocytes in a group of patients at completion of pelvic irradiation (64 Gy). The results of this 'new patient group' is also pooled with those presented in Figs 2 and 3. Two lymphocyte: target cell ratios are presented. Mean log_{10} per cent values ± SE are shown. Pre-irradiation values are put as 2.000 (log_{10} of 100 per cent)

	New patient group		Pooled results	
K 562 cells	12.5 : 1	25 : 1	12.5 : 1	25 : 1
Before irradiation	2.000 (0.105±0.019)* (n=20)	2.000 (0.164±0.020) (n=20)	2.000 (0.105±0.011) (n=42)	2.000 (0.172±0.016) (n=42)
After irradiation	2.17±0.105 (147%)** NS	2.140±0.094 (138%) NS	2.106±0.065 (128%) NS	2.124±0.064 (133%) NS
Chang cells	25 : 1	50 : 1	25 : 1	50 : 1
Before irradiation	2.000 (0.053±0.006) (n=19)	2.000 (0.093±0.013) (n=19)	2.000 (0.061±0.006) (n=40)	2.000 (0.101±0.010) (n=40)
After irradiation	2.156±0.075 (143%) p<0.05	2.177±0.088 (150%) NS	2.041±0.054 (110%) NS	2.060±0.066 (115%) NS

* Absolute cytotoxic index ± SE.
** Antilogarithmized values.

cells was observed to be significantly increased during a post-irradiation period of approximately 2 years whereas, after an initial reduction, cytotoxicity against K562 cells remained at the pretreatment level for a period of 29 months (BLOMGREN et coll. 1982 a). The latter results seemed to be in discordance with those of ONSRUD & THORSBY, showing that the relative spontaneous cytotoxicity against K562 cells was increased 3 to 5 years following radiation therapy for endometrial carcinoma.

In the present investigation the spontaneous cytotoxicity of blood lymphocytes following local radiation therapy for bladder carcinoma has been examined. The changes which occurred differed from those obtained in patients irradiated for breast carcinoma in several respects. Cytotoxicity against K562 cells did not exhibit an inital reduction at completion of irradiation (Fig. 2, Table 2) but rather increased and remained elevated during the rest of the observation period of 18 months (Fig. 2). These results thus seem to be in agreement with those of ONSRUD & THORSBY. Although not statistically significant, spontaneous cytotoxicity against Chang cells also seemed to increase following pelvic irradiation (Fig. 3, Table 2) but not to the same extent as after radiation therapy for breast carcinoma (BLOMGREN et coll. 1982 a). A trivial explanation for the different results obtained in the two patient groups could be that mean values were calculated on an arithmetic basis (because of the presence of some zero values) in the patients with breast carcinoma and on a geometric basis in the patients with bladder carcinoma. When zero values in the breast carcinoma group were arbitrarily put as 5 per cent and the geometric mean values were calculated it was observed that the mean relative cytotoxicity, over a period of 3 to 23 months after irradiation, was 160 per cent for Chang cells and 85 per cent for K562 cells at a lymphocyte : target cell ratio of 25 : 1. The corresponding mean values for the patients with bladder carcinoma were 110 per cent for Chang and 140 per cent for K562 cells. Another explanation for the increased spontaneous cytotoxicity in patients irradiated for bladder carcinoma could be that the assays had become more sensitive with time and that the observed increment therefore was not caused by the irradiation. This possibility, however, could be ruled out since healthy controls who were tested

repeatedly during the course of this investigation (1979–1981) did not exhibit an increased spontaneous cytotoxicity against any of the target cells employed (BLOMGREN et coll. 1982 b).

It is thus concluded that the radiation induced changes of spontaneous cytotoxicity of blood lymphocytes differ between patients with breast carcinoma or bladder carcinoma. However, it cannot be stated that pelvic irradiation always results in a relative increase of cytotoxicity since O'TOOLE et coll. (1973) observed that it was reduced against allogeneic bladder carcinoma cells in such patients following radiation therapy. One explanation for this finding could be that different effector cells, such as natural killer cells (NK) and lymphocytes mediating antibody dependent cellular cytotoxicity (ADCC), are involved to a varying extent in the killing of different target cells. Possibly ADCC may contribute more to the killing of bladder carcinoma cells than of Chang and K562 cells in patients with bladder carcinoma. This would fit with the observation that the relative ADCC activity in the blood lymphocyte population is reduced following irradiation for a variety of human tumors located in different regions of the body (WASSERMAN et coll. 1975, 1978, MCCREDIE et coll. 1979).

Another interesting observation made in the present investigation is that the proportion of lymphocytes with FcR for IgG (EA-rosettes) was significantly increased already at termination of pelvic irradiation and remained elevated for at least 18 months (Fig. 1, Table 1). This was in contrast to the situation after irradiation for breast carcinoma in which there was a sharp reduction of the proportion of EA-rosette forming cells at completion of irradiation followed by a recovery without any significant overshoot (BLOMGREN et coll. 1982 a). It is conceivable that the increased spontaneous cytotoxicity following pelvic irradiation can at least in part be explained by the increased proportion of EA-rosette forming lymphocytes, since lymphocytes mediating ADCC and most NK cells possess FcR for IgG (PROSS & BAINES 1977).

Basic knowledge concerning the mechanism by which radiation therapy induces lymphopenia may provide an answer to the question why irradiation of different regions of the body results in different changes of the blood lymphocyte population. As pointed out by EKSTRAND et coll. (1981) the lymphopenia which develops is most probably due to a radiation damage of lymphocytes, most of which are recirculating, present in the blood and lymphoid tissues within the irradiated volume (EKSTRAND et coll.) Various lymphocyte compartments, however, differ widely with respect to cellular composition. For instance, the frequency of T-lymphocytes possessing FcR for IgG (T_G cells) is higher in the blood lymphocyte population and spleen than in other organs such as lymph nodes, thymus and gut and conversely the frequency of lymphocytes with FcR for IgM (T_M cells) is lower in the blood and spleen (TREPEL 1974). Thus, when radiation predominantly damages cells which are resident in lymph nodes, a relatively more marked depletion of T_M cells than T_G cells would be expected in the blood and when the irradiated volume mainly contains blood, a more extensive depletion of T_G cells would be expected. The example may explain the divergent results on the effect of radiation therapy directed to different regions of the body on the frequency of T_M and T_G cells in the blood. The proportion of T_G cells is significantly reduced following radiation therapy for breast carcinoma (PETRINI et coll.) whereas it is unchanged or increased after pelvic irradiation or irradiation for Hodgkin's disease (BLOMGREN et coll. 1980, ROMAGNANI et coll. 1980, EKSTRAND et coll.).

In conclusion, the results obtained in the present investigation strongly support the view that radiation therapy of different parts of the body may change the cellular composition and immunologic reactivity of the blood lymphocyte population in different ways.

SUMMARY

The spontaneous cytotoxicity of blood lymphocytes was examined before and after local radiation therapy (64 Gy) for transitional cell carcinoma of the bladder. The lymphocyte count was reduced to approximately 40 per cent at termination of treatment and remained below the pre-treatment level during a follow-up period of 18 months. The relative spontaneous cytotoxicity for ^{51}Cr-labelled K562 cells, derived from a human myeloid leukemia, increased at completion of irradiation and remained elevated during the entire observation period. The relative cytotoxicity against Chang cells, derived from human liver, also seemed to increase but not to the same extent. The increments were probably due to an increased proportion of spontaneously cytotoxic cells bearing Fc-receptors for IgG which were significantly elevated after treatment. It was concluded that the changes differ from those occurring after local irradiation for breast carcinoma. The proportion of lymphoid tissue in relation to the blood volume which is included in the irradiated field may determine the changes of the blood lymphocyte population.

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