ACTA RADIOLOGICA

OFFICIAL ORGAN OF THE RADIOLOGICAL SOCIETIES OF
DENMARK, FINLAND, NORWAY AND SWEDEN

Vol. 13
Fasc. 3

THERAPY PHYSICS BIOLOGY

1974
June

BLOOD LYMPHOCYTES AFTER RADIATION THERAPY OF MAMMARY CARCINOMA

H. BLOMGREN, ULLA GLAS, B. MELÉN and J. WASSERMAN

It has become increasingly evident that the lymphoid system in human subjects may build up a defence against autochthonous tumours. It has been demonstrated that antibodies are produced in some tumour-bearing individuals that are directed against certain structures present in the malignant cells (KLEIN et coll. 1967, MORTON et coll. 1968, EILBER & MORTON 1970, GIRALDO et coll. 1971). Moreover, lymphoid cells obtained from subjects with various types of malignant tumours have been held to be able to inhibit in vitro growth of the specific type of tumour cells and such lymphoid cells have also exhibited a more or less specific cytotoxic activity on the former in vitro (HELLSTRÖM et coll. 1968 a, b, BUBENIK et coll. 1970, DIEHL et coll. 1971, HELLSTRÖM et coll. 1971).

The immune response elicited against a subject's own tumour cells must be considered to be relatively weak and only rarely does the immune defence of the host cause regression of a solid palpable tumour (See EVERSON & COLE 1966). However, it is possible that immunity against a tumour may be of great importance after the mass has been reduced to a minimum by various methods so that the ratio of specifically sensitized lymphocytes to tumour target cells is increased. It is therefore desirable that the clinical method used for removing or reducing the tumour cell mass does not interfere negatively with the lymphoid

From Radiumhemmet, Karolinska sjukhuset, S-104 01 Stockholm, and the Central Microbiologic Laboratory, Stockholm County Council, S-101 22 Stockholm, Sweden. Submitted for publication 14 August 1973.

cell population of the host. Such a negative side effect is well recognized after treatment with various types of cytotoxic drugs (HERSH & OPPENHEIN 1967, CARDOZO 1970), and has also been reported after local radiation therapy of carcinoma (STJERNSWÄRD et coll. 1972). There is reason to assume that immune response against autochthonous tumours which causes destruction of the neoplastic cells or inhibition of their proliferation is mainly via the thymus dependent lymphocyte population (T cells) (MILLER et coll. 1964, GRANT et coll. 1965). Recent reports have suggested however that the non-thymus processed lymphocytes (B cells) may also, in a specific way, destroy tumour cells (LAMON et coll. 1972, O'TOOLE et coll. 1973). See ROITT et coll. 1969 for review of T and B cells.) Attempts have therefore been made to investigate whether the lymphocyte depletion in peripheral blood caused by radiation therapy in carcinoma may be due to a reduction in the number of T or B cells, or both. The use of various markers for T and B lymphocytes has suggested that the lymphocyte depletion following irradiation may be mainly due to a diminished number of T cells (STJERNSWÄRD et coll.).

A detailed analysis of the peripheral blood lymphocyte population before and after radiation therapy of carcinoma of the breast is now presented.

Materials and Methods. Twelve women, aged 52 to 66, with mammary carcinoma were examined. The primary diagnosis was obtained by fine needle aspiration biopsy (FRANZÉN & ZAJICEK 1968). The patients are all included in a clinical trial at present proceeding at Radiumhemmet. In summary, at the time of diagnosis, patients with operable carcinoma of the breast are randomized into three different groups receiving the following types of treatment: (1) radical mastectomy only, (2) radical mastectomy followed by local irradiation, (3) local irradiation followed by radical mastectomy. Healthy subjects, matched for age with the affected patients, served as controls.

Treatment was given with ^{60}Co gamma irradiation or high energy electrons and the doses were planned individually. Postoperative irradiation treatment was directed against the axilla, infra- and supraclavicular regions, both retrosternal regions and the chest wall with preoperative irradiation to the same regions and the breast; the target dose was always 4 500 rad over a period of 6 to 7 weeks.

The total number of lymphocytes per μl blood was calculated from conventional counts of the number in a hemocytometer and determinations of the frequency of various cell types on air-dried blood smears by May-Grünwald and Giemsa staining.

Human lymphocytes which unspecifically bind sheep erythrocytes to their cell membranes (E rosette-forming cells) are considered to be of thymus origin.

Lymphocytes having cell surface bound receptors for activated complement C'3 are labelled as nonthymus derived (EAC rosette-forming cells). (For references see discussion.) The frequency of these cell types in the peripheral blood lymphocyte population was established before and after local radiation therapy in human subjects.

Lymphocytes for rosette formation were obtained by gelatin sedimentation from defibrinated blood without heparin, with subsequent filtration through a nylon wool column (PERLMANN & PERLMANN 1970) followed by centrifugation through a layer of ficoll-isopaque (BÖYUM 1968). The cells prepared in this way regularly contained at least 99 per cent of lymphocytes. The preparation of lymphocytes employed for stimulation tests was performed in the same manner except that the filtration through nylon wool was omitted. The cells thus contained significantly more monocytes than the cell suspensions used for rosette formation.

The experimental procedure for E rosettes (spontaneous rosettes of sheep red blood cells with human lymphocytes) will be described. Rosette formation tests were performed by the method described by JONDAL et coll. (1972) with certain modifications. Sheep red blood cells (SRBC) stored at + 4° C in Alsevers solution were washed three times with tris-buffered Hanks balanced salt solution (HTS) and adjusted to a 0.5 per cent suspension (approximately 75×10^6 cells/ml) in HTS, 0.25 ml of this suspension being mixed with 1×10^6 lymphocytes in 0.25 ml HTS. The mixed cells were incubated for 15 min at 37° C, centrifuged at 200 g for 5 minutes in a refrigerated centrifuge (+ 4° C) and incubated overnight at + 4° C. The pellet was then gently resuspended and one drop of the cell suspension placed on a glass slide, covered with a cover slip, and sealed; 200 lymphocytes were counted and lymphocytes binding 3 SRBC or more were regarded as positive.

The experimental procedure for EAC rosettes (erythrocyte-antibody-complement binding cells) was as follows. SRBC stored and washed as described above were adjusted to a 5 per cent solution in HTS; 5 ml of this suspension were mixed with an equal volume of a rabbit anti-sheep red cell serum diluted 1 : 2 000. This serum was prepared by injecting SRBC, inactivated before use, into a rabbit. The optimal dilution of the serum was determined in preliminary experiments. The mixture of anti-SRBC serum and SRBC was incubated at 37° C for 1 hour, washed three times with HTS and resuspended in the same volume; 5 ml of active human serum (complement) diluted 1 : 10 were then added and the mixture incubated again at 37° C. After 1 hour the cells were washed three times and adjusted to a 1 per cent suspension, 0.25 ml of which was mixed with 0.25 ml HTS containing 1×10^6 lymphocytes. The cells were spun at 200 g for 5 min and incubated for 30 min at 37° C; they were then resuspended with the help

Table 1

Number of lymphocytes per μl blood and frequency of EAC and E rosette-forming cells before and after preoperative, local radiation therapy of mammary carcinoma in 6 patients

	Mean ± SE	
	Before	After
Lymphocytes per μl	1861 ± 268	880 ± 218 ($0.01 > p > 0.005$)*
EAC cells (%)	20.0 ± 2.7	9.5 ± 1.5 ($p < 0.005$)
E cells (%)	57.7 ± 3.4	60.9 ± 2.6 ($0.3 < p < 0.4$)

* Statistical significance from Student's t-test between the values obtained before and after therapy

Table 2

Number of lymphocytes per μl blood and frequency of EAC and E rosette-forming cells before and after postoperative local therapy in 6 patients

	Mean ± SE	
	Before	After
Lymphocytes per μl	2001 ± 160	896 ± 221 ($p < 0.005$)*
EAC cells (%)	16.0 ± 2.8	8.3 ± 2.7 ($0.05 > p > 0.025$)
E cells (%)	48.2 ± 4.9	55.2 ± 1.7 ($p = 0.05$)

* Statistical significance from Student's t-test between the values obtained before and after therapy

Table 3

Pooled data of Tables 1 and 2

	Mean ± SE	
	Before	After
Lymphocytes per μl	1913 ± 170	886 ± 149 ($p < 0.005$)*
EAC cells (%)	18.0 ± 2.0	9.2 ± 1.5 ($p < 0.005$)
E cells (%)	52.9 ± 3.2	58.4 ± 1.7 ($0.025 > p > 0.01$)

* Statistical significance from Student's t-test between values obtained before and after therapy

Table 4

Frequency of EAC and E rosette-forming lymphocytes of 6 healthy controls tested on two occasions with a 1 to 3 month interval

	Mean ± SE	
	Test I	Test II
EAC cells (%)	20.0 ± 3.8	21.0 ± 3.9 ($p > 0.4$)*
E cells (%)	56.3 ± 4.1	58.8 ± 2.9 ($p > 0.3$)

* Statistical significance from Student's t-test between the two test occasions

of a whirl mixer. The counting of EAC rosettes was carried out as described for E rosettes. Every experiment included a control performed in exactly the same way as stated above except that no complement was added to the incubation mixture.

Lymphocyte stimulation tests were performed by suspensions of lymphocytes being washed three times in HTS by centrifugation, counted in a hemocytometer and suspended in Eagle's medium supplemented with glutamine, penicillin and streptomycin. The medium also contained 15 per cent of heat inactivated human serum. Varying numbers of lymphocytes, suspended in 0.5 ml of this medium, were then pipetted into conical bottomed, 15 ml screw cap tubes. The mitogens, dissolved in the same medium, were then added in a volume of 0.5 ml. Phytohaemagglutinin (PHA, Wellcome) was used in a final concentration of 0.5 μg/ml and poke weed mitogen (PWM, Grand Island Biological Comp., N.Y.) at a final concentration of 0.1 μg/ml. Tubes without mitogens served as controls. The total volume of the incubation mixture was always 1.0 ml and all cell concentrations were set up in duplicate. The tubes were closed loosely with caps and incubated at 37° C in a humified 5 per cent CO_2 atmosphere; after 72 hours 0.1 μCi ^{14}C-thymidine (specific activity 54 mCi/mmol) was added to each tube. (For details concerning culture conditions and measurements of ^{14}C-thymidine uptake, see the preceding paper (GLAS & WASSERMAN 1974).)

Results

The frequency of blood lymphocytes forming E and EAC rosettes following radiation therapy was investigated. Purified blood lymphocytes obtained from 12 individual patients were investigated for the frequency of E and EAC rosette-forming cells before and within four weeks after radiation therapy. Half the patients were treated preoperatively and half of them postoperatively.

Table 1 indicates that preoperative irradiation significantly reduced the number of lymphoid cells in the blood. The proportion of EAC rosette-forming cells diminished significantly whereas the frequency of E-binding cells remained largely unchanged. Patients receiving therapy postoperatively (Table 2) also had a significant decrease in lymphocyte counts and in the frequency of EAC rosette-forming cells after irradiation. The proportion of 'E cells' in these patients exhibited a slight, but significant increase. The pooled data from both groups of patients are presented in Table 3. A closer analysis of the individual tests revealed that the therapy caused a decrease in the number of lymphocytes in all tested patients. The frequency of 'EAC cells' decreased in 10 patients, remained constant in 1 and was increased in 1 patient. In contrast, the E rosette-forming cells decreased in proportion in 2 patients, remained unchanged in 1 and increased in 9 patients.

The lymphocytes of healthy controls, matched for age with the carcinoma patients, were similarly examined with regard to the frequency of EAC and E rosette-forming cells. Each was tested on two occasions with an interval of 1 to 3 months. Table 4 indicates that the frequency of the two cell types failed to differ significantly between the two tests; nor did they vary to any degree from the values obtained in the carcinoma patients before therapy ($p > 0.3$ for EAC and $p > 0.2$ for E rosette-forming cells).

The mitogen responses of blood lymphocytes following radiation therapy were investigated. The results already presented revealed that the therapy reduced the number of blood lymphocytes by approximately 50 per cent and that lymphocytes having receptors for activated C'3 decrease proportionally more than the cells binding SRBC. Further to characterize the composition of the lymphocyte pool before and after the therapy, 6 of the patients tested were also examined for the response of their blood lymphocytes to PHA and PWM in vitro.

Preliminary tests disclosed that the PHA response of 0.5×10^6 blood lymphocytes, obtained from the same healthy individual, varied considerably on repetitive testing. The differences in absolute thymidine incorporations, which exceeded a factor of 3, were considered not to reflect variations in the composition of the lymphocyte population tested, but rather to be due to unknown differences in the culture conditions of the cells. For this reason, the mitogen responsiveness of the lymphoid cells from the diseased patients was always expressed in relation to the response of a healthy control whose lymphocytes were cultured at the same time. Each carcinoma patient was examined twice, once before and once after treatment. Lymphocytes from the same normal donor served as control cells on both these occasions. It was thus assumed that the response of the cells from the healthy subjects remained constant before and after the therapy of those with carcinoma.

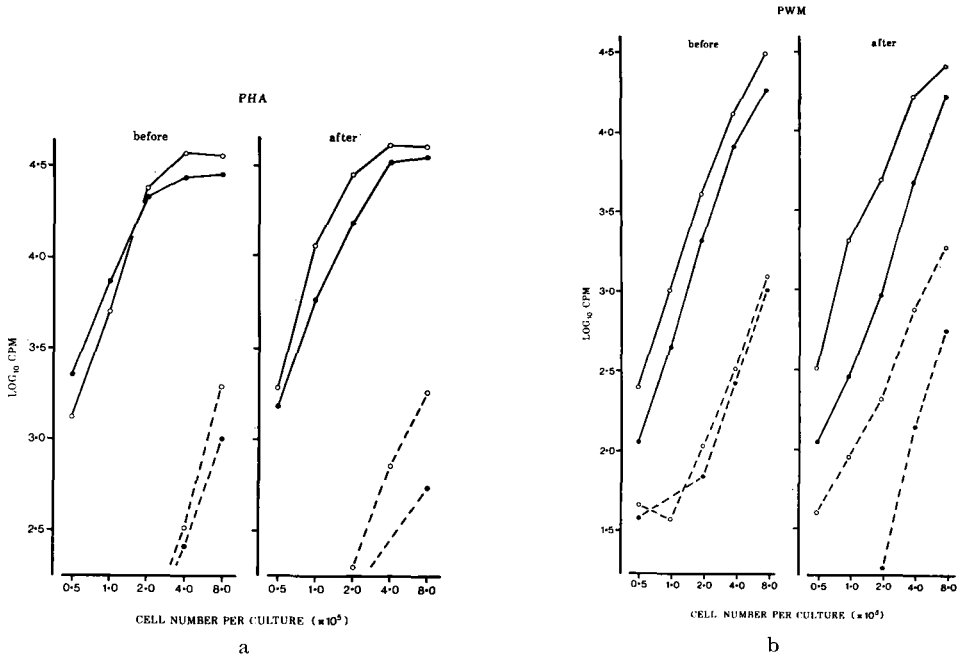

Fig. 1. ^{14}C-thymidine incorporations by various numbers of blood lymphocytes obtained before and after therapy in carcinoma and a control. The lymphocytes were stimulated by a) PHA and b) PWM. O———O Lymphocytes of diseased patient with and O- - -O without stimulant, ●———● Lymphocytes of control with and ●- - -● without stimulant.

Fig. 1 serves to illustrate a test of the in vitro responsiveness, expressed as $\log_{10}$ counts per minute (cpm) of incorporated ^{14}C-thymidine of lymphoid cells to PHA and PWM as a function of cell number. It can be seen that the PHA response increased in an almost linear fashion on increasing the number of cultured cells from 0.5×10^5 up to 2.0×10^5. A further increase in cell concentration resulted in a flattening off of the curves. Moreover, this particular test indicated that the PHA responsiveness of the lymphocytes from the patient with carcinoma increased after therapy, assuming that the true reactivity of the control lymphocytes remained at the same level. With PWM as a mitogen an almost linear increase in thymidine incorporation was obtained in the dose range of 0.5 to 8.0×10^5 cells per ml of culture medium. It is obvious that in this test the PWM response of the lymphocytes from the patient increased after irradiation.

The mitogen responses of the lymphoid cells of the individual patient before and after therapy, are presented in Fig. 2. Isotope incorporation, obtained by varying numbers of cultured cells, is expressed as a percentage of cpm incorporated in parallel cultures containing the corresponding number of lymphoid cells

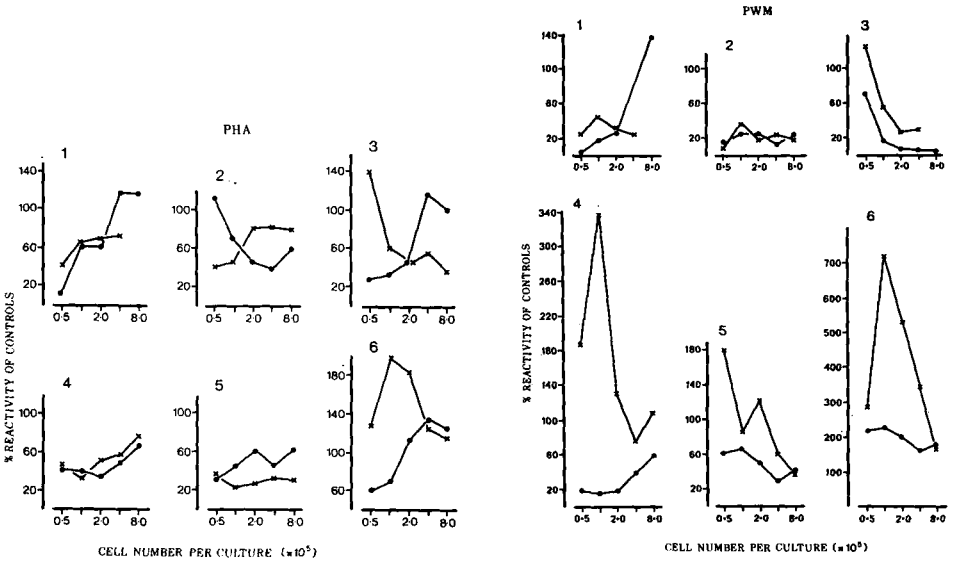

Fig. 2. a) PHA and b) PWM responses of the lymphocytes in 6 patients with carcinoma of the breast before and after radiation therapy. The cells from each patient were cultivated at five different cell concentrations. Symbols represent the thymidine uptakes expressed as percentages of the values for the lymphocytes of the controls. Cases 2, 3 and 4 received preoperative and Cases 1, 5 and 6 postoperative therapy. Thymidine incorporations in Case 6 are illustrated in Fig. 1 a. ●———● Values obtained before and ×———× after radiation therapy.

from the healthy controls. The PHA response of the lymphocytes of the affected patients is markedly lower than that of the controls. This difference is best observed, with one exception, with low concentrations of cultured cells. The PHA responsiveness of the cell populations varied following treatment. An important observation is that the change in responsiveness was highly dependent on the number of cells per culture. Thus, the lymphocytes of the same patient could exhibit an increased PHA reactivity when small numbers of cells, and a decreased reactivity when large numbers of cells, were cultured. Similar results were obtained with PWM as a stimulant. The responses of the lymphocytes before treatment of the affected patients were markedly lower, with one exception, than those of the controls. The PWM response of the lymphocytes following treatment increased, in 2 patients most markedly. This enhanced reactivity was most conspicuous with low doses of cultured cells.

The pooled data, illustrated in Fig. 2, are presented in Tables 5 and 6, respectively. It is obvious that the PHA responsiveness of the lymphoid cells from the

Table 5

PHA responsiveness of various numbers of blood lymphocytes obtained before and after radiation therapy. ^{14}C-thymidine incorporations by the cell cultures are related to the values obtained in corresponding cultures from the controls. Mean values, expressed as percentages of controls calculated from the data presented in Fig. 3

Cell number per culture ($x10^5$)	Mean $\pm$ SE	
	Before	After
0.5	48 ± 14 ($0.01 > p > 0.005$)*	71 ± 20 ($p = 0.2$)**
1.0	53 ± 6 ($p < 0.005$)	70 ± 27 ($0.3 > p > 0.2$)
2.0	60 ± 12 ($0.01 > p > 0.005$)	76 ± 23 ($0.2 > p > 0.1$)
4.0	83 ± 18 ($0.2 > p > 0.1$)	71 ± 13 ($0.2 > p > 0.1$)
8.0	89 ± 13 ($p > 0.2$)	67 ± 15 ($0.2 > p > 0.1$)

* Statistical significance from Student's t-test between the values obtained before therapy and the corresponding values obtained for controls

** Statistical significance between the values obtained before and after therapy

Table 6

PWM responsiveness of various numbers of blood lymphocytes obtained before and after radiation therapy. ^{14}C-thymidine incorporations by the cell cultures are related to the values obtained in corresponding cultures from the controls. Mean values, expressed as percentages of controls, calculated from the data presented in Fig. 4

Cell number per culture ($x10^5$)	Mean $\pm$ SE	
	Before	After
0.5	65 ± 32 ($p > 0.1$)*	133 ± 44 ($0.05 > p > 0.0025$)*
1.0	62 ± 34 ($p > 0.1$)	214 ± 114 ($0.3 > p > 0.2$)
2.0	54 ± 30 ($0.1 > p > 0.05$)	144 ± 80 ($p > 0.4$)
4.0	49 ± 30 ($0.1 > p > 0.05$)	93 ± 51 ($0.3 > p > 0.2$)
8.0	73 ± 27 ($p > 0.2$)	81 ± 33 ($p > 0.4$)

* Same as in Table 5.

diseased patients before therapy was significantly lower than that of the healthy controls. This impaired reactivity was best observed with small concentrations of cultured cells. Following irradiation, the responsiveness increased by 0.5 to 2.0×10^5 cells per culture and there was a slight decrease with higher cell numbers. However, neither of these effects, when presented in this manner, was statistically significant. The mean PWM responses of the cell populations mentioned failed to differ significantly from those of the controls.

Table 7

Changes of the PHA and PWM responses of the lymphocytes following radiation therapy. The values are expressed as percentages of the values, in relation to the controls, obtained before the therapy. Mean values calculated from the data presented in Figs 3 and 4

Cell number per culture ($x10^5$)	Mean ± SE	
	PHA	PWM
0.5	210 ± 68 ($0.025 > p > 0.01$)*	333 ± 107 ($0.1 > p > 0.05$)*
1.0	129 ± 38 ($p > 0.2$)	532 ± 297 ($p > 0.1$)
2.0	125 ± 20 ($0.1 > p > 0.05$)	300 ± 93 ($0.1 > p > 0.05$)
4.0	102 ± 25 ($p > 0.4$)	258 ± 62 ($0.05 > p > 0.025$)
8.0	85 ± 19 ($p > 0.2$)	112 ± 24 ($p > 0.3$)

* Statistical significance from Student's t-test between values obtained before (100 %) and after the therapy, with PHA or PWM respectively as stimulants

Enhanced responsiveness after radiation therapy was evident for this mitogen. The data are also presented in Table 7 in which the responses of the lymphoid cells after therapy are expressed as percentages of the values obtained before treatment. This manner of presentation demonstrated that the responsiveness to PHA but not to PWM increased significantly following therapy with the lowest concentration of cultured cells.

Discussion

The present investigation was undertaken primarily to examine the changes in the peripheral blood lymphocyte population following local therapy of patients with mammary carcinoma. Closer knowledge of the side effects on the lymphoid apparatus caused by therapy may be of much importance since the immune defence may be disturbed. The aim of the therapy may thus be lost and the patients may be rendered more susceptible to infection.

The results of the present work have clearly demonstrated that the lymphocyte pool in peripheral blood is markedly reduced by local therapy, in agreement with the results of other investigators (Goswitz et coll. 1963, Meyer & Sayre 1970, McCredie et coll. 1972, Stjernswärd et coll.). In an attempt to characterize any possible changes in the composition of the lymphocyte population, four different markers, more or less specific for various types of lymphocytes were used: (1) The frequency of lymphocytes having the capacity to bind SRBC unspecifically to their cell membranes (E cells). Strong support has arisen for the view

that, in the human subject, these are T cells (BRAIN et coll. 1970, BRAIN & GORDON 1971, JONDAL et coll.); (2) The frequency of lymphocytes bearing receptors for activated C'3 (EAC) being considered as non-T cells. Considerable evidence exists that B cells possess these receptor structures (BIANCO et coll. 1970, MICHLMAYR & HUBER 1970, DUKOR et coll. 1971, JONDAL et coll.) although it is possible that this specific receptor is not entirely restricted to this type of lymphocyte. Recent data indicate that another cell type, probably neither a T nor B lymphocyte, designated a 'null-lymphocyte' (GREENBERG et coll. 1973), also possesses this type of receptor (WIGZELL & PERLMANN, personal communication); (3) Responses of lymphocyte populations in vitro to PHA. This mitogen stimulates predominantly T cells as well as lymphoid cells bearing membrane bound γ globulins, presumably B cells (PHILLIPS & ROITT 1973); (4) Responses of lymphocyte populations in vitro to PWM. This agent seems to stimulate B cells as wells as T cells (CHESSIN et coll. 1966, DOUGLAS et coll. 1967). The two former tests identify the different lymphocyte sub-populations whereas the latter tend to reflect changes in the relative frequency of cells capable of being triggered to increased DNA synthesis by these mitogens. The stimulation tests also revealed the 'health' of the lymphocytes which are potentially capable of blast transformation in response.

The results demonstrate that the frequency of E and EAC rosette-forming lymphocytes in the patients with carcinoma fails to differ significantly from the values obtained in the controls. However, the responsiveness of lymphocytes from the former to PHA proved to be significantly lower than that of cells obtained from controls. This difference was most evident when the responses of small numbers of cells per culture were employed; an impaired mitogen responsiveness of such lymphocytes has been confirmed by GARRIOCH et coll. (1970), WHITTAKER et coll. (1970), DUCOS et coll. (1970), HANN & TAKITA (1972), and LANDER & BONE (1973) but denied by ROBINSON & HURVITZ (1966), RICCI et coll. (1966), ROBERTS (1970), SUTHERLAND et coll. (1971), and FISHER et coll. (1972). These discrepancies in the results may be explained by different lymphocyte culture conditions; the relationship between the cell number and the response to the mitogens support this view. The importance of an optimal concentration of the stimulant has also been stressed by other investigators (DUCOS et coll., LANDER & BONE). No difference in PWM responsiveness between patients with carcinoma and the normal controls was evident.

The irradiation of patients with carcinoma decreased the frequency of EAC rosette-forming lymphocytes significantly whereas the proportion of E rosette-forming cells (mainly T cells) exhibited a slight, but definite increase. Such changes were not observed in the controls during the same time period. These results are in some disagreement with those obtained by STJERNSWÄRD et coll.

who reported the reverse effect after the treatment of patients with mammary carcinoma. However, this apparent discrepancy may be explained by the fact that the frequency of contaminant nonlymphoid cells, such as monocytes, differed in the two investigations. During the present preparation of the lymphocyte suspensions, the cells were passed through a nylon column to remove any others that might be adhering whereas such a step was apparently not included in the tests described by STJERNSWÄRD et coll.

A number of reports have appeared in which the PHA response of lymphocytes from the peripheral blood was examined before and after the therapy of malignancy; some of the investigators reported decreased responsiveness following irradiation (MILLARD 1965, THOMAS et coll. 1971, ILBERY et coll. 1971, STJERNSWÄRD et coll.) whereas others recorded an increase (MCCREDIE et coll. 1972). The present results may offer some explanation for these contradictions. In general, the lymphocytes of the same subject exhibited an increased responsiveness both to PHA and PWM following irradiation when small concentrations of cultured cells were used and a decrease at higher cell concentrations. The results of the statistical analysis however do not permit more than a suggestion that therapy of a malignant lesion may enhance the mitogen responsiveness of lymphocytes and that this effect can only be expressed when small numbers of cells per culture are employed. One explanation for these cell dose dependent results may be that the optimal number of mitogen molecules for triggering the lymphocytes to DNA synthesis is not the same for cells subjected to irradiation. The number of mitogen molecules per cell theoretically available with small numbers of lymphocytes per culture is then higher than when large numbers of cells are used. Thus, lymphocytes which require many PHA or PWM molecules to be triggered increase in proportion after therapy either due to enrichment of such cells or to radiation induced changes in the activation threshold of the lymphocytes. Another explanation may be that the nutrients present in the culture medium permit only optimal cell metabolism for a very limited number of lymphocytes; and furthermore that a high cell number per culture, containing the same volume of medium, results in an exhaustion of the nutrients during the first few days of culture and causes subsequent decreased cell activity. This could theoretically create a situation in which small numbers of responsive lymphocytes might yield higher thymidine incorporations per unit cell number on the fourth and fifth days of incubation than larger cell numbers.

In conclusion, the present results have demonstrated that the local therapy of patients with carcinoma of the breast causes a depletion in the pool of lymphocytes in the blood. The frequency of EAC rosette-forming cells decreases and that of E rosette-forming cells increases. The lymphocyte stimulation tests with unspecific mitogens suggest an increased reactivity of the lymphocyte population

following irradiation. However, this effect is dose dependent and seems to hold true only for low lymphocyte concentrations. The increased responsiveness of lymphocytes can be demonstrated only if variations in culture conditions are taken into consideration. The relevance of these findings to the in vivo situation remains to be elucidated. In any event a large proportion of blood lymphocytes following therapy appear to remain and be capable of being activated and possibly reacting against foreign structures on malignant cells. Such an immune response has been suggested to operate in at least some cases (MOORE & FOOT 1949, BERG 1959, CUTLER et coll. 1969, HAMLIN 1968, CHAN et coll. 1971).

Acknowledgements

The authors wish to thank Professor J. Einhorn for his criticism of the manuscript, Mrs Sirkka Nygren and Mr Ricardo Giscombe for their technical assistance and Mr Ahmed Mohi el Din for statistical evaluation of the results. The investigation was supported by grants from the King Gustav V Jubilee Fund and the Swedish Cancer Society.

SUMMARY

Peripheral blood lymphocytes in mammary carcinoma were examined before and after radiation therapy. The proportion of those binding sheep erythrocytes (thymus-dependent cells) or those having receptors for activated complement (thymus-independent cells) was similar to that of healthy controls. The treatment produced lymphopenia and the proportion of thymus-independent lymphocytes decreased, although that of the thymus-dependent cells increased. The results suggest enhanced mitogen responsiveness with low cell concentrations and impaired reaction of cultures containing those that are higher.

ZUSAMMENFASSUNG

Vor und nach Bestrahlung wurde eine Untersuchung der Lymphozyten im peripheren Kreislauf vorgenommen. Die Proportion solcher Lymphozyten, die Schaferythrozyten zu binden vermochten (thymusabhängige Zellen) und solcher die Rezeptoren für aktiviertes Komplement enthielten (thymusunabhängige Zellen) war dieselbe wie für normale Kontrollen. Die Strahlenbehandlung rief eine Lymphopenie vor und das Verhältnis von thymusunabhängigen Zellen nahm ab, obwohl das Gegenteil für die thymusabhängigen Zellen zutraf. Die Resultate zeigen, dass niedrige Konzentrationen eine erhöhte Reaktion auf Mitogen aufweisen, jedoch umgekehrt hohe Zellkonzentrationen in Kulturen nur eine geringe Reaktion.

RÉSUMÉ

Les auteurs ont examiné avant et après traitement par les radiations les lymphocytes du sang périphérique dans le cancer du sein. La proportion de ceux qui se fixent aux érythrocytes du mouton (cellules thymo-dépendantes) ou ceux qui ont des récepteurs pour le com-

plément activé (cellules thymo-indépendantes) sont semblables à celles des sujets témoins sains. Le traitement entraîne une lymphopénie et la proportion des lymphocytes thymo-indépendants diminue alors que celle des cellules thymo-dépendantes augmente. Ces résultats font penser que les faibles concentrations cellulaires s'accompagnent d'une capacité de réponse mitogène augmentée et que les cultures contenant une plus forte concentration cellulaire ont une réaction diminuée.

REFERENCES

BERG J. W.: Inflammation and prognosis in breast cancer. A search for host resistance. Cancer 12 (1959), 714.

BIANCO C., PATRICK R. and NUSSENZWEIG V.: A population of lymphocytes bearing a membrance receptor for antigen-antibody-complement complexes. I. Separation and characterization. J. exp. Med. 132 (1970), 702.

BLOOM H. J. G. and FIELD J. R.: Impact of tumor grade and host resistance and survival of women with breast cancer. Cancer 28 (1971), 1580.

BÖYUM A.: Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation. Scand. J. clin. Lab. Invest. 21 (1968), Suppl. 27.

BRAIN P. and GORDON J.: Rosette formation by peripheral lymphocytes. II. Inhibition of the phenomenon. Clin. exp. Immunol. 8 (1971), 441.

— — and WILLETTS W. A.: Rosette formation by peripheral lymphocytes. Clin. exp. Immunol. 8 (1970), 1.

BUBENIK J., PERLMANN P., HELMSTEIN K. and MOBERGER G.: Immune response to urinary bladder tumours in man. Int. J. Cancer. 5 (1970), 39.

CARDOZO E. L.: The effect of cytostatic therapy with cyclophosphamide alone or in combination with metotrexate on the photohaemagglutinin lymphocyte culture and on the immunoglobulins. Oncology 24 (1970), 261.

CHAN S. P., MACA R. D., LEVINE P. H. and TING R. C.: Immunological studies of human breast cancer. I. Serum reactivity against a lymphoid cell line (Belev) derived from a breast cancer patient as detected by complement fixation test. J. nat. Cancer. Inst. 47 (1971), 511.

CHESSIN L. N., BÖRJESON J., WELSH P. D., DOUGLAS S. D. and COOPER H. L.: Studies on human peripheral blood lymphocytes in vitro. J. exp. Med. 124 (1966), 873.

CUTLER S. J., BLACK M. M., MORK T., HARVEI S. and FREEMAN C.: Further observations on prognostic factors in cancer of the female breast. Cancer 24 (1969), 653.

DIEHL V., JEREB B., STJERNSWÄRD J., O'TOOLE C. and ÅSTRÖM L.: Cellular immunity to nephroblastoma. Int. J. Cancer 7 (1971), 277.

DOUGLAS S. D., HOFFMAN P. F., BÖRJESON J. and CHESSIN L. N.: Studies on human peripheral blood lymphocytes *in vitro*. III. Fine structural features of lymphocyte transformation by poke weed mitogen. J. Immunol. 98 (1967), 17.

DUCOS J., MIQUERES J., COLOMBIES P., KESSONS A. and POUJOULET N.: Lymphocyte response to PHA in patients with lung cancer. Lancet 1 (1970), 1111.

DUKOR P., BIANCO C. and NUSSENZWEIG V.: Bone marrow origin of complement receptor lymphocytes. Europ. J. Immunol. 1 (1971), 491.

EILBER F. R. and MORTON D. L.: Sarcoma-specific antigens: Detection by complement fixation with serum from sarcoma patients. J. nat. Cancer Inst. 44 (1970), 651.

EVERSON T. C. and COLE W. H.: Spontaneous regression of cancer. Saunders, Philadelphia 1966.

FISHER B., SAFFER E. A. and FISHER E. R.: Studies concerning the regional lymph nodes in cancer. Cancer 30 (1972), 1202.

FRANZÉN S. and ZAJICEK J.: Aspiration biopsy in diagnosis of palpable lesions of the breast. Acta radiol. Ther. Phys. Biol. 7 (1968), 241.

GARRIOCH D. B., GOOD R. A. and GATTI R. A.: Lymphocyte response to PHA in patients with non-lymphoid tumours. Lancet 1 (1970), 618.

GIRALDO G., BETH E. and HIRSHAUT L.: Human sarcomas in culture. Foci of altered cells and a common antigen; induction of foci and antigen in human fibroblast cultures by filtrates. J. exp. Med. 133 (1971), 454.

GLAS U. and WASSERMAN J.: Effect of radiation treatment on cell-mediated immune response in carcinoma of the breast. Acta radiol. Ther. Phys. Biol. 13 (1974), 83.

GOSWITZ F. A., ANDREWS G. A. and KNISELEY R. M.: Effects of local irradiation (Co^{60} teletherapy) on the peripheral blood and bone marrow. Blood 21 (1963), 605.

GRANT G. A. and MILLER J. F. A. P.: Effect of neonatal thymectomy on induction of sarcomata in C57Bl mice. Nature 205 (1965), 1124.

GREENBERG A. H., HUDSON L., SHEN L. and ROITT I. M.: Antibody-dependent cell-mediated cytotoxicity due to a 'null' lymphoid cell. Nature New Biology 242 (1973), 111.

HAMLIN I. M. E.: Possible host resistance in carcinoma of the breast: A histological study. Brit. J. Cancer 22 (1968), 34.

HANN T. and TAKITA H.: Immunological impairment in bronchogenic carcinoma: A study of lymphocyte response to phytohaemagglutinin. Cancer N. Y. 30 (1972), 616.

HELLSTRÖM I., HELLSTRÖM K. E., PIERCE G. E. and BILL A. H. (a): Demonstration of cell-bound and humoral immunity against neuroblastoma cells. Proc. nat. Acad. Sci. (Wash.), 60 (1968), 1231.

— — — and YANG J. P. S. (b): Demonstration of cell-bound and humoral immunity to different types of neoplasms. Nature (Lond.), 220 (1968), 1352.

— — and SJÖGREN H.: Demonstration of cell-mediated immunity to human neoplasms of various histological types. Int. J. Cancer 7 (1971), 1.

HERSH E. M. and OPPENHEIM J. J.: Inhibition of *in vitro* lymphocyte transformation during chemotherapy in man. Cancer Res. 27 (1967), 98.

ILBERY P. L. T., RICKINSON A. B. and THRUM C. E.: Blood lymphocyte replicating ability as a measurement of radiation dosage. Brit. J. Radiol. 44 (1971), 834.

JONDAL M., HOLM G. and WIGZELL H.: Surface markers on human T and B lymphocytes. J. exp. Med. 136 (1972), 207.

KLEIN G., CLIFFORD P., KLEIN E., SMITH R. T., MINOWADA J., KOURILSKY F. M. and BURCHENAL J. H.: Membrane immunofluorescence reactions of Burkitt lymphoma cells from biopsy specimens and tissue culture. J. nat. Cancer Inst. 39 (1967), 1027.

LAMON E. W., SKURZAK H. M., KLEIN E. and WIGZELL H.: *In vitro* cytotoxicity by a non-thymus-processed lymphocyte population with specificity for a virally determined tumor cell surface antigen. J. exp. Med. 136 (1972), 1072.

LANDER I. and BONE G.: Lymphocyte transformation in large bowel cancer. Brit. J. Cancer 27 (1973), 409.

MCCREDIE J. A., INCH W. R. and SUTHERLAND R. M.: Effect of postoperative radiotherapy on peripheral blood lymphocytes in patients with carcinoma of the breast. Cancer 29 (1972), 349.

MEYER K. K. and SAYRE P.: Radiation-induced lymphocyte immune deficiency. Arch. Surg. 101 (1970), 114.

MICHLMAYR G. and HUBER H.: Receptor sites for complement on certain human peripheral blood lymphocytes. J. Immunol. 105 (1970), 670.

MILLARD R. E.: Effect of previous irradiation on the transformation of blood lymphocytes. J. clin. Path. 18 (1965), 783.

MILLER J. F. A. P., TING R. C. and LAW I. W.: Influence of thymectomy on tumor induction by polioma virus in C57B1 mice. Proc. Soc. exp. Biol. 116 (1964), 323.

MOORE O. S. and FOOT F. W.: The relatively favorable prognosis of medullary carcinoma of the breast. Cancer 2 (1949), 635.

MORTON D. L., MALMGREN R. A., HOLMES E. C. and KETCHAM A. S.: Demonstration of antibodies against human malignant melanoma by immunofluorescence. Surgery 64 (1968), 233.

O'TOOLE C., PERLMANN P., WIGZELL H., UNSGAARD B. and ZETTERLUND C.: Lymphocyte cytotoxicity in bladder cancer. No requirement for thymus-derived cells? Lancet (1973), p. 1085.

PERLMANN P. and PERLMANN H.: Contactual lysis of antibody coated chicken erythrocytes by purified lymphocytes. Cell. Immunol. 1 (1970), 300.

PHILLIPS B. and ROITT I. M.: Evidence for transformation of human B lymphocytes by PHA. Nature New Biology 241 (1973), 254.

RICCI M., PASSALEVA A. and RICCA M.: Mixed lymphocyte reaction in cancer. Lancet 2 (1966), 503.

ROBERTS M. M.: Lymphocyte transformation in breast cancer. Brit. J. Surg. 57 (1970), 381.

ROBINSON E. and HURWITZ D.: In vitro studies of lymphocytes from cancer patients. Israel J. med. Sci. 2 (1966), 80.

ROITT I. M., GREAVES M. F., TORRIGLANTI G., BROSTOFF J. and PLAYFAIR J. H.: The cellular basis of immunological responses. Lancet 2 (1969), 367.

STJERNSWÄRD J., JONDAL M., VANKY F., WIGZELL H. and SEALY R.: Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma. Lancet 24 (1972), 1352.

SUTHERLAND R. M., INCH W. R. and MCCREDIE J. A.: Phytohaemagglutinin (PHA)-induced transformation of lymphocytes from patients with cancer. Cancer 27 (1971), 574.

THOMAS J. W., COY P., LEWIS H. S. and YUEN A.: The effect of therapeutic irradiation on lymphocyte transformation in lung cancer. Cancer 27 (1971), 1046.

WHITTAKER M. G., REES K. and CLARK C. G.: Reduced lymphocyte transformation in breast cancer. Lancet 1 (1970), 892.