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Reduction of PWM-Induced Ig Secretion by Blood Lymphocytes Following Local Radiation Therapy for Breast Cancer*

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Abstract—*The PWM* in vitro *induced Ig secretion by blood lymphocytes was examined in* 11 *women before and after local postoperative radiation therapy* (45.0 Gy) *for breast cancer. It was observed that both the IgM and IgG secretions were significantly reduced at completion of irradiation and remained below the pretreatment levels* 3 months *after irradiation. IgM secretion was reduced to the highest extent.*

INTRODUCTION

MANY investigators have observed a reduction of the size of the peripheral T-cell pool and an impaired immunological reactivity following local radiation therapy employing *in vitro* and *in vivo* tests which mainly measure the reactivity of T-cells [1–7]. The influence of radiation therapy on the peripheral B-cell pool is less extensively studied. Using various markers to identify B-cells it has been concluded that the size of this lymphocyte subpopulation is also decreased after irradiation but there are contradictory results as to whether the T- or B-cells are depleted to the highest relative extent [2, 3, 8–10]. Moreover, to our knowledge, tests revealing the functional activity of the B-cell subset after local radiation therapy have not been reported.

We have examined the capacity of PWM-exposed peripheral lymphocytes to secrete Ig, a B-lymphocyte function, before and after radiation therapy for breast cancer.

MATERIALS AND METHODS

Patients

Eleven women with primary operable breast cancer are presented in this study. Their ages ranged from 44 to 75 years with a mean age of 59. Preoperative diagnosis was obtained by fine needle aspiration biopsy [11].

Treatment of the patients

After having undergone a modified radical mastectomy all the patients in whom histopathological examination of the surgical specimen had demonstrated tumor involvement of the axillary nodes and/or primary tumors exceeding 3 cm in diameter received postoperative radiation therapy. Four to six weeks after surgery the operated area of the chest wall was irradiated using a 6–9 MeV electron beam. The internal mammary and the supraclavicular regions were irradiated using an interior ^{60}Co portal. The target dose was calculated at a depth of 3 cm. The axillary region was irradiated by an anterior and posterior ^{60}Co field. A dose of 45.0 Gy (4500 rad) was delivered to all regions in approximately 5 weeks.

Blood sampling

Heparinized venous blood was obtained within 1 week before irradiation was started, on the last day of radiation therapy and 12–16 weeks after completion of the treatment. These blood samples were used for determinations of leukocyte counts with differentials and for *in vitro* tests (see below).

Separation of lymphoid cells

Lymphoid cells were separated by centrifugation of the blood on Ficoll–Isopaque [12].

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The cell suspensions were then washed four times by centrifugation in a balanced salt solution (BSS) and thereafter twice in RPMI 1640 medium. The cell preparations were not depleted of phagocytic cells.

Mitogen

Vials of poke weed mitogen (PWM) were purchased from Grand Island Biological Co., Grand Island, NY. The same batch was used for all tests. The contents of each vial were dissolved in 5 ml of distilled water. Lymphocytes were stimulated with PWM at final dilutions of 1:300 and 1:3000.

Lymphocyte cultures

A slight modification of the technique described by Wasserman *et al.* was used [13]. Lymphocytes (5×10^5) were cultured in glass tubes containing 1 ml of RPMI 1640 medium supplemented with glutamine, antibiotics and 10% of heat inactivated fetal calf serum (56°C for 30 min). Two batches were used. Sera from the same batch were used for the tests of each individual patient. The experimental cultures were incubated with PWM whereas the controls did not receive any stimulant. All cultures were set up in duplicate. After seven days of incubation at 37°C in a 5% CO_2–air atmosphere the tubes were centrifuged, the supernatants collected and stored at −80°C.

Determination of Ig concentrations

The amounts of IgG and IgM in the culture supernatants were determined by an enzyme-linked immunosorbent assay (ELISA). The technique, which is a modification of the method of Voller *et al.* [14], has been described before [13]. All the supernatants obtained from each patient were thawed and tested on the same day using the same reagents. The concentrations of Ig are expressed in μg/100 ml. Variability within the duplicates usually did not exceed 10%.

Data processing and statistical evaluation

The aim of the present investigation was to examine whether Ig-secretion by peripheral lymphocytes changes following radiation therapy. To be able to detect a decrease we have selected those 11 patients who exhibited increased Ig release upon cultivation with PWM in the first sample. In three patients PWM failed to increase Ig secretion and they remained nonresponsive in the two subsequent tests. These patients are thus not included in this presentation. The initial value of each patient was set at 100% and the values of the two subsequent tests are related to this value and also expressed as a percentage. Statistical evaluation was performed using the Student's *t*-test.

RESULTS

At completion of radiation therapy the number of lymphocytes per μl of blood decreased to 28 ± 14% and three months later there was an increase to 48 ± 24% of the pretreatment value (means ± S.D.)

Table 1 shows that the IgM release increased by stimulating the lymphocytes with PWM and that there was an extensive variation between the patients before irradiation (44 ± 64 μg/100 ml). These individual variations, however, were of the same order of magnitude throughout the study, which indicates that there were no major fluctuations of the measurements performed. IgM secretion was reduced to 12–21% at termination of radiation therapy. Three months later it was still significantly diminished but some recovery seemed to have taken place. The spontaneous IgM release, in the absence of PWM, was reduced in nine patients and there was a high relative increase in two at completion of irradiation.

Table 2 shows that PWM also increased the IgG release of the lymphocytes and that there was a high variability between the patients before irradiation (58 ± 53 μg/100 ml). IgG release of the PWM-stimulated cultures was reduced at completion of irradiation and remained below the pretreatment level 3 months later. The reductions, however, were not as extensive as for IgM and were statistically significant only when the lowest PWM concentration was employed. Spontaneous IgG release did not exhibit any consistent changes after irradiation.

DISCUSSION

The results of this study have shown that the PWM-induced IgM secretion of lymphoid cells is sharply reduced following local radiation therapy and that there seems to be a slight recovery 3 months later. IgG secretion was also reduced, but to a lesser extent. It should be emphasized that only the blood lymphocyte population has been examined and the conclusion cannot be extended to include other lymphoid compartments, such as those residing in the spleen and lymph nodes. It is uncertain whether the reduced Ig secretion of the blood lymphocyte population has any clinical significance, since the serum

Table 1. *IgM release of blood lymphoid cells* in vitro *before and after radiation therapy*

PWM conc. (%)	Before irradiation	At completion of radiation therapy: % of pre-treatment value	At completion of radiation therapy: Decrease* Increase	Three months following radiation therapy: %of pre-treatment value	Three months following radiation therapy: Decrease Increase
0	100 (44±64)†	100±196 NS	9/2	409±1105 NS	8/3
1:300	100 (275±182)	21±24 $P<0.01$	11/0	58±61 $P<0.05$	8/3
1:3000	100 (229±140)	12±14 $P<0.001$	11/0	24±23 $P<0.001$	11/0

The cells were cultured without or with two different concentrations of PWM. The values are expressed as a percentage of the pretreatment value. Means±S.D. are shown.

*Number of patients in whom IgM secretion was numerically decreased compared to the pretreatment value/number of patients in whom IgM secretion increased.

†Mean absolute values ± S.D. expressed as μg/100 ml.

Table 2. *IgG release of blood lymphoid cells* in vitro *before and after radiation therapy*

PWM conc. (%)	Before irradiation	At completion of radiation therapy: % of pre-treatment value	At completion of radiation therapy: Decrease* Increase	Three months following radiation therapy: % of pre-treatment value	Three months following radiation therapy: Decrease Increase
0	100 (58±53)†	186±192 NS	6/5	144±87 NS	5/6
1:300	100 (213±211)	67±95 NS	9/2	88±104 NS	9/2
1:3000	100 (230±166)	58±92 $P<0.05$	9/2	38±34 $P<0.005$	10/1

The cells were cultured without or with two different concentrations of PWM. The values are expressed as a percentage of the pretreatment value. Means±S.D. are shown.

*Number of patients in whom IgG secretion was numerically decreased compared to the pretreatment value/number of patients in whom IgG secretion was increased.

†Mean absolute values±S.D. expressed as μg/100 ml.

Ig levels and antiviral antibody titres are not significantly reduced in breast cancer patients treated with local radiation [2, 15].

There may be several explanations for the diminished PWM-induced Ig secretion following radiation therapy. Theoretically it could be due to a reduced frequency of Ig-secreting B-cells after irradiation or a reduced frequency of function of those T-cells which act as helper cells during the PWM-induced Ig secretion [16]. Another possibility is that the frequency of suppressor cells is increased after irradiation. It is unlikely that there is an increased frequency of suppressor T-cells since they have been observed to be more radiosensitive than the T-helper cells [13, 17–19]. Another possibility is that the reduced PWM stimulation is due to an increased proportion of inhibitory monocytes following radiation therapy. Such cells can largely explain the reduced reactivity of blood lymphocytes in the mixed lymphocyte culture [20] and their proliferative responses to PPD-tuberculin after irradiation [21]. Possibly the decrease can be explained by both a reduced frequency of Ig-secreting cells and inhibitory monocytes. Experiments will be performed to elucidate these questions.

REFERENCES

1. Glas U, Wasserman J. Effect of radiation treatment on cell-mediated immune response in carcinoma of the breast. *Acta Radiol Ther Phys Biol* 1974; **13:** 83.
2. Blomgren H, Berg R, Wasserman J, Glas U. Effect of radiotherapy on blood lymphocyte population in mammary carcinoma. *Int J Radiat Oncol Biol Phys* 1976; **1:** 177.
3. Stjernswärd J, Jondal M, Vanky F, Wigzell H, Sealy R. Lymphopenia and change in distribution of human B and T lymphocytes in peripheral blood induced by irradiation for mammary carcinoma. *Lancet* 1972; **24:** 1352.
4. Blomgren H, Wasserman J, Edsmyr F, Baral E, Petrini B. Reduction of responder and stimulator capacities of peripheral lymphoid cells in the mixed lymphocyte culture following external radiotherapy. *Int J Radiat Oncol Biol Phys* 1977; **2:** 297.
5. Check JH, Damsker JI, Brady LW, O'Neill EA. Effect of radiation therapy of mumps-delayed type hypersensitivity reaction in lymphoma and carcinoma patients. *Cancer* 1973; **32:** 580.
6. Cosimi AB, Brunstetter FH, Kemmerer WT, Miller BN. Cellular immune competence of breast cancer patients receiving radiotherapy. *Arch Surg* 1973; **107:** 531.
7. Nordman E, Toivanen A. Effect of radiation on the immune function in patients with mammary, pulmonary or head and neck carcinoma. *Acta Radiol Oncol* 1978; **17:** 3.
8. Petrini B, Wasserman J, Blomgren H, Baral E. Blood lymphocyte subpopulations in breast cancer patients following radiotherapy. *Clin Exp Immunol* 1977; **29:** 36.
9. Raben M, Walach N, Galili Y, Schlesinger M. The effect of radiation therapy on lymphocyte subpopulations in cancer patients. *Cancer* 1976; **37:** 1417.
10. Campbell AC, Wiernik G, Wood J, Hersey P, Waller CA, MacLennan ICM. Characteristics of the lymphopenia induced by radiotherapy. *Clin Exp Immunol* 1976; **23:** 200.
11. Franzén S, Zajicek J. Aspiration biopsy in diagnosis of palpable lesions of the breast. *Acta Radiol Ther Phys Biol* 1968; **7:** 241.
12. Jondal M, Holm G, Wigzell H. Surface markers on human T and B lymphocytes. *J Exp Med* 1972; **136:** 207.
13. Wasserman J, von Stedingk L-V, Biberfeld G, Petrini B, Blomgren H, Baral E. The effect of irradiation on T-cell suppression of ELISA determined Ig production by human blood B cells *in vitro*. *Clin Exp Immunol* 1979; **38:** 366.
14. Voller A, Bidwell DE, Bartlett A. Enzyme immunoassays in diagnostic medicine, theory and practice. *Bull WHO* 1976; **53:** 55.
15. Pettingale KW, Merrett TG, Tee DEH. Prognostic value of serum levels of immunoglobulins (IgG, IgA, IgM and IgE) in breast cancer: A preliminary study. *Br J Cancer* 1977; **36:** 550.
16. Janossy G, Greaves M. Functional analysis of murine and human B-lymphocyte subsets. *Transplant Rev* 1975; **24:** 177.
17. Moretta L, Webb SR, Grossi CE, Lydyard PM, Cooper MD. Functional analysis of two human T-cell subpopulations: Help and suppression of B-cell responses by T-cells bearing receptors for IgM or IgG. *J Exp Med* 1977; **146:** 184.
18. Fauci AS, Pratt KR, Whalen G. Activation of human B-lymphocytes. VIII. Differential radiosensitivity of subpopulations of lymphoid cells involved in the polyclonally-induced PFC responses of peripheral blood. B lymphocytes. *Immunology* 1978; **35:** 715.
19. Siegal FP, Siegal M. Enhancement by irradiated T-cells of human plasma cell production. Dissection of helper and suppressor functions *in vitro*. *J Immunol* 1977; **118:** 642.
20. Blomgren H, Wasserman J, Wallgren S, Ideström K, Baral E, Petrini B. Changes in mixed lymphocyte culture (MLC) functions of peripheral lymphoid cells after radiation therapy for breast cancer. *Int J Radiat Oncol Biol Phys* 1979; **5:** 49.
21. Blomgren H, Wasserman J, Baral E, Petrini B. Evidence for the appearance of nonspecific suppressor cells in the blood after local radiation therapy. *Int J Radiat Oncol Biol Phys* 1978; **4:** 249.