FROM THE DEPARTMENT OF GYNECOLOGIC ONCOLOGY THE NORWEGIAN RADIUM HOSPITAL, AND THE INSTITUTE OF TRANSPLANTATION IMMUNOLOGY, THE UNIVERSITY OF OSLO, OSLO, NORWAY.

EARLY STAGE CARCINOMA OF THE UTERINE CERVIX

Effects of intracavitary radium treatment on lymphoid cells in blood and pelvic lymph nodes

M. ONSRUD, I. GRAHM and G. GAUDERNACK

Abstract

Sixteen patients with early stage carcinoma of the uterine cervix treated with primary radical hysterectomy and pelvic lymphadenectomy were compared with 17 patients who four to six weeks before the operation received intracavitary treatment with radium. The calculated radiation dose to the pelvic wall was approximately 10 Gy. The distribution of lymphoid cells in blood and pelvic lymph nodes was studied by an indirect immunoflourescence technique using monoclonal antibodies. The radium treated group showed a significant reduction of circulating OKT4+ (T helper) and OKT8+ (T suppressor/cytotoxic) lymphocytes. The number of Leu7+ (natural killer) cells and 1D5+ cells (monocytes) was not changed, but the ratio between monocytes and T cells was increased after radium therapy. In cell suspensions obtained from the pelvic lymph nodes, the radium treatment induced a significant reduction of the OKT4+ cell fraction. It is concluded that this low dose rate regimen of intracavitary treatment induces changes in the immune system which are of the same type as those seen after external field irradiation.

Key words: Radiation, injurious effect; hematopoietic; immunity.

Immunosuppression is an unwanted side effect of radiation therapy. After external field irradiation, long-lasting depressions of T lymphocyte numbers and of T cell responses have been found (8, 12, 19). Radiation-induced immunosuppression is further characterized by aberrations in the distribution of T cell subsets (18). Monocytes and natural killer cells seem to be more radioresistant than T cells (12).

The immunosuppressive effect of intracavitary radiation treatment is little known. In a recent study on patients operated upon for early stage cervical carcinoma, we found that blood T cells from patients pretreated with intracavitary radium had retained their antigen specific responses, whereas the responses of lymph node T cells were depressed (14).

The hybridoma-produced monoclonal antibodies OKT4 and OKT8 are considered to characterize mainly the T helper and the T suppressor/cytotoxic cells, respectively (16); and the OKT4/OKT8 ratio is a commonly used parameter for the competence of the T cell immune system. Monocytes are identified by the monoclonal antibody 1D5 (9), and NK cells by the Leu7 antibody (1). By the use of these antibodies and an indirect immunoflourescence technique, our purpose was to study the distribution of lymphoid cells in blood and pelvic lymph nodes of patients operated upon for early stage cervical carcinoma who had, or had not, received preoperative radium treatment.

Material and Methods

Patients. Sixteen patients with small tumors localized to the ectocervix were operated primarily. Seventeen patients with larger tumors received preoperative radium insertions according to a modified Paris method (10). The treatment was given during a ten-day period and delivered 60 to 70 Gy to point A and 8 to 15 Gy to point B. Four to six weeks later, a radical hysterectomy with pelvic lymphadenectomy was carried out, as described by KOLBENSTVEDT & KOLSTAD (10). In 29 of the cases, lymph node biopsies for immunologic study could be taken from the region along the obturator nerve. One or two nodes were dissected free of fatty tissue, weighed, and brought to the laboratory for immediate handling. Preoperative lymphography and peroperative palpation gave no evidence for

Accepted for publication 24 January 1986.

Table 1

Distribution of the patients with cervical carcinoma according to age, histology and stage (FIGO classification)

	No pre-operative radiation treatment (n=16)	Pre-operative radium treatment (n=17)
Age (years)		
Median	32	42
Range	(25–53)	(27–64)
Histology		
Squamous cell carcinoma	14	10
Adenocarcinoma	2	6
Adenosquamous carcinoma	–	1
Stage (FIGO)		
I a	7	–
I b	9	16
II a	–	1
Microscopic lymph node metastasis	2	4

Table 2

White blood cell counts in patients with cervical cancer treated or not treated with intracavitary radium. Median numbers per 1×10^{-9} and ranges

Cell population	No radiation treatment (n=16)	Preoperative radium treatment (n=17)
Total leucocytes	5.6 (3.0–11.7)	4.8 (2.6–8.9)
Granulocytes	3.5 (1.9–6.1)	3.2 (1.0–4.5)
Lymphocytes	1.9 (0.8–2.5)	1.2* (0.7–1.8)

* The value is significantly different from that of the control group, p<0.005.

node metastasis, but a microscopic metastasis was detected in 6 of the cases (21%). Charcteristics of the two patient groups are shown in Table 1.

Cell separation and surface markers. Venous blood with EDTA as an anticoagulant was taken immediately before the operation in the morning, and total leucocyte and differential counts performed. Mononuclear cells were obtained by Ficoll-Isopaque flotation (5). The node biopsies were finely minced in RPMI 1640 medium and filtered through a stainless steel mesh, as previously described (14). All cell handling was performed at 0–4°C. The T cell subsets were identified by the monoclonal antibodies OKT4 and OKT8 (Ortho Pharmaceuticals, Raritan, New Jersey, USA), monocytes by the monoclonal antibody 1D5 (9), and NK cells by the Leu7 antibody (Becton Dickinson, Mechelen, Belgium). The binding of antibody to viable cells was detected by FITC-conjugated sheep anti-mouse lg (N 1031, Amersham, England). Cells incubated with second layer antibody only served as negative controls. The cells were examined for flourescence in a phase-contrast microscope equipped with an epifluorescence condenser. To determine percentages, at least 200 cells were counted. Wilcoxon's rank sum test was used for statistical evaluation of the difference between groups of results.

Table 3

Absolute numbers of circulating lymphoid cell subsets as determined by the monoclonal antibodies OKT4 (T helper cells), OKT8 (T suppressor/cytotoxic cells), 1D5 (monocytes) and Leu7 (natural killer cells) in patients treated or not treated with intracavitary radium. Median numbers per 1×10^{-6} and ranges

Cell population	No radiation treatment (n=16)	Preoperative radium treatment (n=17)
OKT4+ cells	956 (551–1 435)	660* (390–1 047)
OKT8+ cells	504 (258–1 468)	415* (216–749)
Leu7+ cells	222 (69–396)	209 (126–260)
1D5+ cells	288 (154–808)	304 (184–843)

* Values significantly different from those of the control group, p<0.005.

Results

Blood leucocytes. The white blood cell counts, as determined by total leucocyte and differential counts, are shown in Table 2. The number of circulating lymphocytes was significantly reduced in the patient group treated with intracavitary radium.

Subpopulations of blood mononuclear cells. After radium treatment, the numbers of circulating OKT4+ (T helper) and OKT8+ (T suppressor/cytotoxic) cells were significantly reduced (Table 3). The OKT4/OKT8 ratios were, however, similar in the two treatment groups (median values being 1.5 and 1.6 in the radium-treated group and in the control group, respectively). The numbers of Leu7+ (NK) cells and 1D5+ cells (monocytes) were not significantly different in the two treatment groups. When individual calculations of the ratio between monocyte numbers (1D5+ cells) and T lymphocyte numbers (OKT4+ cells plus OKT8+ cells) were done, the radium-treated group showed a significantly higher level as compared with the controls (Table 4).

Lymph node cells. The median numbers of cells recov-

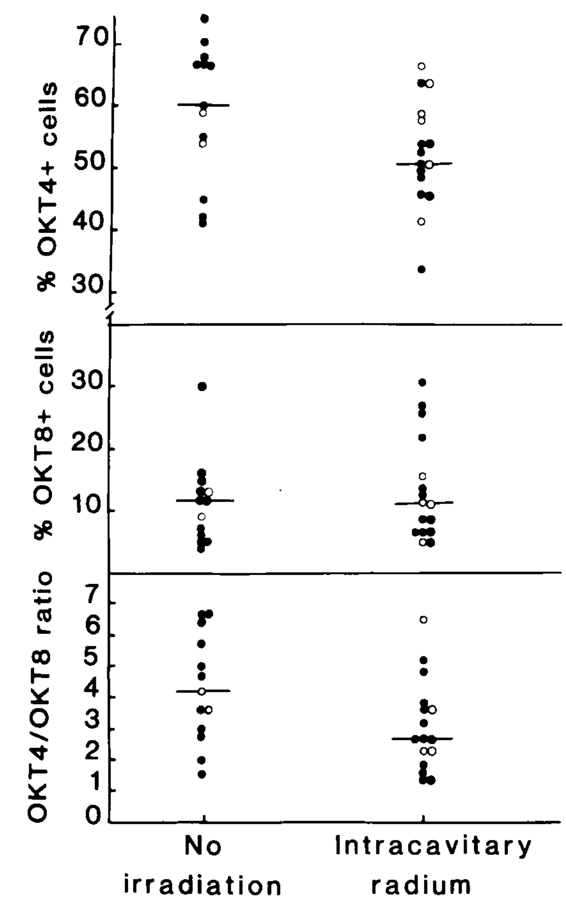

Fig. 1. The fraction of OKT4+ (T helper) cells, OKT8+ (T suppressor/cytotoxic) cells, and the OKT4/OKT8 ratio in cell suspensions from pelvic lymph nodes of patients operated primarily and in patients receiving preoperative intracavitary radium treatment. Open symbols signify patients with microscopic node metastasis.

Table 4

The ratio between blood monocytes (as determined by 1D5+ cells) and blood T cells (as determined by the sum of OKT4+ cells and OKT8+ cells) in patients treated or not treated with intracavity radium

	No radiation treatment (n=16)	Preoperative radium treatment (n=17)
Median	0.27	0.35*
Range	0.08–0.52	0.18–0.58

* Significantly different from control value, $p<0.005$.

ered from the pelvic lymph nodes of the radium-treated and the non-treated patients were 29×10^6 and 80×10^6, respectively. There were great inter-individual variations, and the difference between the groups was of borderline significance ($0.10>p>0.05$). The node cell suspensions from radium-treated patients contained a significantly lower fraction of OKT4+ cells than those of the control patients (median values being 51% and 60%, $p=0.04$) (Figure). No difference was seen in the OKT8+ cell fraction. The OKT4/OKT8 ratios of the two patient groups were 2.8 and 4.2, respectively ($p=0.05$). The node cell suspensions contained few 1D5+ cells (1–12%) and Leu7+ cells (0–2%), and their numbers wer not related to the treatment given.

Discussion

The T lymphocytes are highly sensitive to irradiation. For various T cell responses, D_0 values of 0.5 to 2.3 Gy are reported (2). In patients receiving external radiation therapy for various types of cancer, a marked lymphopenia is found shortly after treatment (4, 8, 12, 19). Depressed numbers of circulating T helper cells are observed as long as 10 years after the therapy (18). The side effects of intracavitary radiation treatment has received less attention. The present study on patients undergoing radium treatment shows that 4 to 6 weeks after therapy, a significant depression of circulating T cells is found. For how long a period this depression persists is unknown. In peripheral blood, both OKT4+ and OKT8+ cells were affected, whereas in the regional lymph nodes only the OKT4+ cell fraction was significantly reduced.

This study and those of others (11, 15) have shown that the relative content of OKT4+ cells is higher in lymph nodes than in peripheral blood; and localized node irradiation would therefore mainly affect the OKT4+ cell pool. Rapid and long-lasting depressions of T cell numbers and T cell responses are observed after endolymphatic administration of ^{32}P colloids (17). Experimental studies on rats have shown that 5 Gy of irradiation causes a total depletion of local node lymphocytes; but the nodes are rapidly repopulated by recirculating lymphocytes (6). Intracavitary radium therapy, as used in our regimen, gives a radiation dose of 8 to 15 Gy to the nodes on the pelvic wall (10). This dose should be sufficient to deplete the nodes of radiosensitive T cells (and B cells). During the 4 to 6 weeks period before operation, the nodes are probably repopulated by T cells recirculating from peripheral blood. This hypothesis may explain the marked depression of blood T cells (both OKT4+ and OKT8+ cells) and the minimal depletion of lymph node T cells (mainly OKT4+ cells) seen after radium treatment.

The lymph nodes should be considered not only as privileged sites for metastases, but also as important reservoirs for immunologic active cells. Recently, we found higher antigen-specific responses of lymph node T cells than of blood T cells (14). The higher OKT4+/OKT8+ ratio of the node cells probably explains this difference. It is unknown whether the reduction of this ratio, as seen after radium therapy, is of any clinical significance. Whether the lymphoid cells of the nodes play any role in preventing metastasis is also unclear. In cervical cancer, a predominant lymphocytic pattern of the pelvic nodes has been correlated to a decreased frequency of node metastasis, lower recurrence rate, and increased survival (20). In nodes affected by malignant lymphoma, the OKT4/OKT8 ratio is depressed (8). In our study, the patients who had microscopic node metastasis did not differ in any particular way from those without metastasis (Figure). The biopsies were, however, taken at random from non-suspicious nodes. The results might have been different if metastatic nodes had been studied separately. In that case, however, the aberrations could have been a consequense of the metastatic process rather than a reason for it.

The NK cells are currently receiving much attention in tumor immunology. As determined by the Leu7 monoclonal antibody, intracavitary radium treatment did not influence the number of NK cells in peripheral blood.

In the radium-treated patients, increased monocyte/T cell ratios of peripheral blood were observed. (Table 4). These findings are in accordance with those of BLOMGREN et coll. (4) who studied breast cancer patients treated by external radiation. In our study, the increased monocyte/T cell ratio seemed to be due to decreased numbers of T cells rather than increased monocyte numbers. Immunosuppression caused by monocytes has been described in several types of cancer. In a recent study on patients with cervical carcinoma, we observed a potentiation of T cell responses to microbial antigens after removal of monocytes by adherence on gelatin/fibronectin coated surfaces (3). This type of immunosuppression is presumably of greater importance in patients presenting high monocyte/T cell ratios. The prognostic significance of this phenomenon is unclear.

The results of this study indicate that the effects on the immune system of intracavitary radium treatment are of the same type as those seen after external field radiation.

ACKNOWLEDGEMENT

This study was supported by grants from the Norwegian Society for Fighting Cancer.

REFERENCES

1. ABO T. and BALCH C. M.: A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J. Immunol. 127 (1981), 1024.
2. ANDERSON R. E. and WARNER N. L.: Ionizing radiation and the immune response. Adv. Immunol. 24 (1976), 215.
3. BJERCKE S., ONSRUD M. and GAUDERNACK G.: Suppressive effect of monocytes in patients with carcinoma of the uterine cervix. Acta Obstet. Gynecol. Scand. (in press)
4. BLOMGREN H., WASSERMAN J., BARAL E. and PETRINI B.: Evidence for the appearance of non-specific suppressor cells in the blood after local radiation therapy. Int. J. Radiat. Oncol. Biol. Phys. 4 (1978), 249.
5. BØYUM A.: Isolation of lymphocytes, granulocytes and macrophages. Scand. J. Immunol. (1976) Suppl. No. 5, p. 9.
6. ENGESET A.: Local irradiation of lymph nodes in rat. Morphological and functional alterations with relation to cancer therapy. Progr. Exp. Tumor Res. 8 (1966), 225.
7. FORD W. L.: Lymphocyte migration and immune responses. Progr. Allergy 19 (1975), 59.
8. HEIER H. E., CHRISTENSEN I., FRØLAND S. and ENGESET A.: Early and late effects of irradiation for seminoma testis on the number of blood lymphocytes and their B and T subpopulations. Lymphology 8 (1975), 69.
9. KAPLAN G. and GAUDERNACK G.: In vitro differentiation of human monocytes. J. Exp. Med. 156 (1982), 1101.
10. KOLBENSTVEDT A. and KOLSTAD P.: The difficulties of complete pelvic lymph node dissections in radical hysterectomy for carcinoma of the cervix. Gynecol. Oncol. 4 (1976), 244.
11. KVALØY S., MARTON P., HØST H., SOLHEIM B. G. and GODAL T.: Distribution of T-cell subsets identified by monoclonal antibodies in cell suspensions from lymph node biopsies of human B-cell lymphomas. Scand. J. Haematol. 28 (1982), 293.
12. ONSRUD M.: Whole pelvic irradiation in stage I endometrial carcinoma. Changes in numbers and reactivities of some blood lymphocyte subpopulations. Gynecol. Oncol. 13 (1982), 282.
13. — and KVALØY S.: Influence of progestogen therapy on T lymphocyte subsets. Acta Path. Microbiol. Immunol. Scand. 92 (1984), 89.
14. — and QVIGSTAD E.: Squamous cell carcinoma of the cervix stage IB. Numbers and reactivities of pelvic lymph node T-cells. Gynecol. Oncol. 20 (1985), 177.
15. POPPEMA S., BKAN A., REINHERZ E. L., MCCLUSKEY R. T. and SCHLOSSMAN S. F.: Distribution of T-cell subsets in human lymph nodes. J. Exp. Med. 153 (1981), 30.
16. REINHERZ E. L., KUNG P. C., GOLDSTEIN G. and SCHLOSSMAN S. F.: Separation of functional subsets of human T-cells by monoclonal antibody. Proc. Natl. Acad. Sci. USA 76 (1979), 4061.
17. RICHTER G., HEIDENBLUTH I. and HEIDELBACH U.: On endolymphatic radionuclide therapy of malignant melanoma and its immunological side effects. Dermatol. Monatsschr. 168 (1982), 797.
18. ROTSEIN S., BLOMGREN H., PETRINI B., WASSERMAN J. and BARAL E.: Long term effects on the immune system following local radiation therapy for breast cancer. I. Cellular composition of the peripheral blood lymphocyte population. Int. J. Radiat. Oncol. Biol. Phys. 11 (1985), 921.
19. STJERNSWÄRD J., JONDAL M., VANKY F., WIGZELL H. and SEARLY R.: Lymphopenia and change in distribution of human B and T-lymphocytes in peripheral blood induced by irradiation. Lancet I (1972), 1352.
20. VAN NAGELL J. R., DONALDSON E. S., PARKER J. C., VAN DYKE A. H. and WOOD E. G.: The prognostic significance of pelvic lymph node morphology in carcinoma of the uterine cervix. Cancer 39 (1977), 2624.