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CHANGES IN LYMPHOID CELL DISTRIBUTION AFTER INTRAPERITONEAL ADMINISTRATION OF ^{32}P COLLOIDS

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Abstract

The effect of intraperitoneal administration of ^{32}P colloids on the distribution of T lymphocyte subpopulations and monocytes was studied using monoclonal antibodies and a flow cytometry technique. Thirty-nine patients with ovarian carcinoma without residual tumor after primary operation were examined either before the administration of 260 to 370 MBq of ^{32}P, 4 to 6 days after therapy, or 4 to 10 months after therapy. A significant reduction of circulating OKT4+ (T helper) cells occurred after therapy, and the reduction lasted throughout the observation period. Monocyte numbers were not significantly changed. It is concluded that intraperitoneal instillation of the ^{32}P isotope may induce the same type of changes in circulating lymphoid cells as those seen after external field irradiation.

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 (10, 14, 22). Radiation-induced immunosuppression is further characterized by aberrations in the distribution of T cell subsets (17). A relative increase in the number of immunosuppressive monocytes after radiation therapy may also be of importance (2).

The lymphopenia seen during and after radiation therapy may have several causes: irradiation of circulating blood, bone marrow, or lymph nodes. HEIER et coll. (10) consider the irradiation of peripheral blood to be most important. Intraperitoneal instillation of ^{32}P colloid causes irradiation of several lymph nodes and lymph vessels with very low doses delivered to blood and bone marrow (3). This gives us an opportunity to study selectively the effect of lymphoid tissue irradiation on blood lymphoid cells in man.

The hybridoma-produced monoclonal antibodies OKT4 and OKT8 are considered to characterize mainly the T helper cells and the T suppressor/cytotoxic cells respectively (20); 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 (12). By the use of these antibodies and a flow cytometry technique, our purpose was to study the distribution of blood lymphoid cells in patients treated with ^{32}P colloids intraperitoneally.

Material and Methods

Patients. The 39 patients examined were under treatment for ovarian carcinoma. In all cases, hysterectomy, bilateral salpingo-oophorectomy and omentectomy had been performed, and there was no macroscopic tumor remaining after surgery. The majority of the patients were operated upon before the referral to our institution. The ^{32}P labelled colloidal suspension was instilled during relaparotomy or, in a few cases, during laparoscopy. The suspen-

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sion contained chromic hydroxide particles with diameters between 0.1 and 1 μm (P-32-C8 from Sorin, Italy), and the amount of ^{32}P given ranged from 260 to 370 MBq depending on the weight of the patients. The radioactive suspension was instilled together with 2 l of physiologic saline. Three groups of individually different patients were examined: 15 patients were examined before ^{32}P instillation; 10 patients were examined 4 to 6 days after ^{32}P instillation; and 14 patients free of recurrence were seen 4 to 10 months after the therapy. Table 1 shows that the distribution according to age, stage of disease (FIGO classification), and histologic type of tumor was fairly even in the 3 groups of patients.

Cell separation, surface markers, and flow cytometry. Venous blood with EDTA as an anticoagulant was taken in the morning and the tests were performed immediately. Total leucocyte and differential counts were done. Mononuclear cells were obtained by Ficoll-Isopaque floatation (4). The cells were washed twice in RPMI 1640 medium supplemented with 3 mg/ml bovine serum albumin. All cell handling was done on ice. An indirect immunofluorescence technique was used for quantitation of lymphoid cell populations, as described earlier (16). Briefly, T cell subsets were identified by the monoclonal antibodies OKT4 and OKT8 (Ortho Pharmaceuticals, Raritan, New Jersey, USA), and monocytes by the monoclonal antibody 1D5 (12). Binding of antibody to cells was detected by FITC-conjugated sheep anti-mouse Ig (N 1031, Amersham, England). Cells incubated with second layer antibody only served as negative controls. The samples were analysed in a model 50-H Cytofluorograph (Ortho Instruments, Westwood, Massachusetts, USA), interfaced with a 2150 computer system. The OKT4+ and OKT8+ fractions were determined as percentages of the total lymphocyte count. The 1D5+ fraction was determined as a percentage of the total mononuclear cell count.

Individual and median results within each group are given, and the difference between groups of results is evaluated statistically by the Wilcoxon's rank sum test.

Table 1

Distribution according to age, stage, and histology in groups of patients with ovarian carcinoma examined before or after intraperitoneal administration of ^{32}P colloids

	Before ^{32}P (n=15)	4–6 days after ^{32}P (n=10)	4–10 months after ^{32}P (n=14)
Age (years)			
Median	54	56	53
Range	26–70	29–72	31–73
Stage (FIGO)			
I	12	9	10
II	2	1	2
III	1	–	2
Histology			
Serous	2	1	4
Mucinous	5	3	4
Endometrioid	2	3	2
Clear-cell	3	2	1
Mixed	2	–	1
Undifferentiated	1	1	2

Table 2

White blood cell counts in groups of operated patients with ovarian carcinoma examined before or after intraperitoneal administration of ^{32}P colloids. Median numbers per $l \times 10^{-9}$ and ranges

Cell population	Before ^{32}P (n=15)	4–6 days after ^{32}P (n=10)	4–10 months after ^{32}P (n=14)
Total leucocytes	5.6 (3.3–9.0)	5.9 (4.9–8.4)	4.9 (2.5–9.5)
Granulocytes	3.0 (1.9–5.1)	3.4 (2.6–6.0)	3.0 (1.4–7.1)
Lymphocytes	1.8 (1.2–3.0)	1.7 (1.0–3.2)	1.5 (0.8–1.9)
Monocytes	0.2 (0.1–0.4)	0.4 (0.1–0.5)	0.3 (0.1–0.5)

Results

Leucocytes. The white blood cell counts, as determined by total leucocyte and differential counts, were not significantly changed after ^{32}P treatment (Table 2). The median number of lymphocytes was slightly reduced in the group examined 4 to 10 months after treatment.

T cell subpopulations. Following ^{32}P treatment, a reduction in the relative content of OKT4+ (T helper) cells occurred (Fig. 1). This decrease was most marked in the group examined 4 to 10 months after therapy (the median value being 35%, compared with 47% before therapy, $p<0.005$). The relative content of OKT8+ (T suppressor/cytotoxic) cells

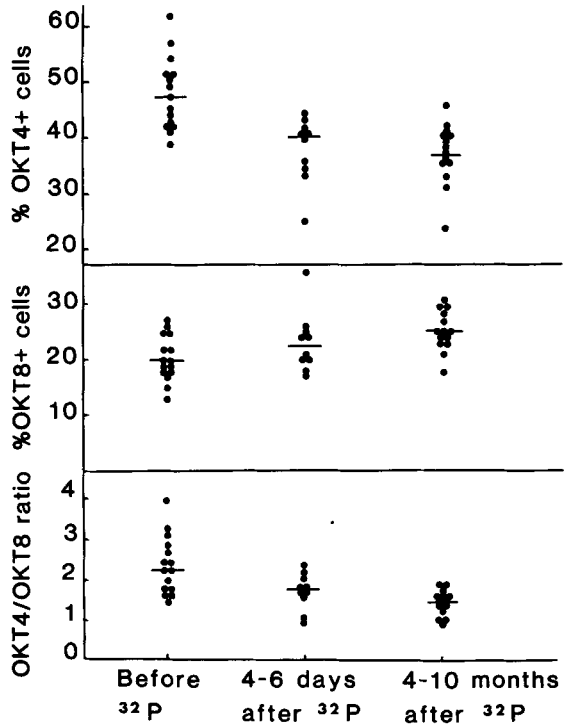

Fig. 1. The fraction of OKT4+ (T helper) cells, OKT8+ (T suppressor/cytotoxic) cells, and the OKT4/OKT8 ratio in blood lymphocytes of radically operated ovarian carcinoma patients examined either before intraperitoneal instillation of ^{32}P colloids, 4–6 days after, or 4–10 months after treatment. Individual and median results for each group are indicated.

Table 3

Absolute numbers of circulating OKT4+ (T helper) cells and OKT8+ (T suppressor/cytotoxic) cells in patient groups examined before or after intraperitoneal administration of ^{32}P colloids. Median numbers per $l \times 10^{-6}$ and ranges

T cell subpopulation	Before ^{32}P (n=15)	4–6 days after ^{32}P (n=10)	4–10 months after ^{32}P (n=14)
OKT4+ cells	826 (474–1 588)	654 (374–1 274)	598* (198–714)
OKT8+ cells	394 (184–571)	340 (207–1 171)	356 (212–497)

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

was slightly increased after ^{32}P treatment, whereas the median OKT4/OKT8 ratio became significantly depressed (from 2.4 to 1.5, $p<0.005$). In terms of absolute numbers, a significant reduction in the OKT4+ cell population was found, whereas the OKT8+ T cell subset remained unchanged (Table 3).

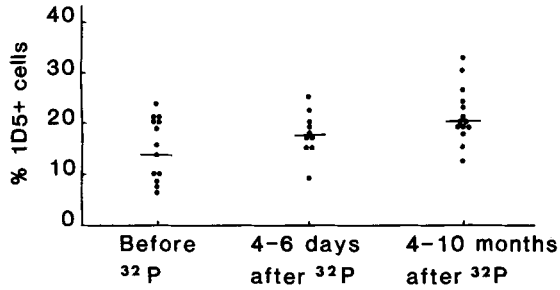

Fig. 2. The fraction of 1D5+ cells (monocytes/macrophages) in blood mononuclear cells from the same patients as in Fig. 1.

Monocytes. The relative number of 1D5+ cells was slightly higher 4 to 10 months after ^{32}P therapy than before therapy (median numbers 20% and 14%, $p=0.05$) (Fig. 2). The absolute number of circulating monocytes was, however, not significantly changed.

Discussion

In ovarian carcinoma, the whole peritoneal cavity is at risk for metastatic spread. In early stages of the disease, subclinical tumor deposits may be found on the diaphragm, on the omentum, and in lymph vessels draining the peritoneal cavity (18). Intraperitoneal administration of radioactive labelled colloid particles is widely used in an attempt to destroy such micrometastases. The preferred isotope is ^{32}P which has a half-life of 14.3 days and emits beta particles with a range of about 4 mm. The overall 5-year survival rate for radically operated ovarian carcinoma is approximately 80 per cent (6). Clearly, there is need for a careful evaluation of both short-term and long-term side effects of any adjuvant treatment given to this patient group.

The side effects of ^{32}P colloids given intraperitoneally are considered to be few. Surgical complications appear much less frequently than after the use of colloidal ^{198}Au (5). The present investigation has shown that intraperitoneal ^{32}P causes immunosuppression by reducing the numbers of circulating OKT4+ cells and thus reducing the OKT4/OKT8 ratio for a period of at least 4 to 10 months. These changes are similar to those found after external field radiation. PETRINI et coll. (17) reported lowered T helper/suppressor ratios for up to 10 years after radiation therapy for breast carcinoma.

T lymphocytes are known to be highly sensitive to radiation. For various T cell responses, D_0 values of 0.5 to 2.3 Gy are reported (1). Doses as low as 0.15

Gy may inhibit the generation of cytotoxic T cells in vivo (9). After intraperitoneal instillation of radiophosphor, the dose delivered from ^{32}P circulating in peripheral blood is estimated to 0.012 Gy, and the dose delivered in the bone marrow 0.06 (3). The surface dose delivered by ^{32}P colloids adherent to the peritoneum was estimated to approximately 30 Gy. It is reasonable to assume that this gives a significant irradiation of lymphoid cells circulating in small vessels beneath the peritoneum.

After intraperitoneal instillation, a part of the colloid particles will be drained from the peritoneal cavity through the lymphatics. Phagocytic cells of the monocyte/macrophage series probably participate in this transportation. The radiation sensitivity of these cells is considered to be low; and some monocyte functions may even be activated by radiation (1). In the present investigation, no significant change in the aboslute number of circulating monocytes was seen. It is unlikely, therefore, that ^{32}P therapy will damage the monocyte/macrophage branch of the immune system.

The accumulation of radioactivity in intrathoracic lymph nodes is clearly demonstrated by gamma camera imaging (3). The cells localized in these nodes may get a significant radiation dose. The relative content of OKT4+ cells is higher in lymph nodes than in peripheral blood (19); and localized node irradiation would therefore mainly affect the OKT4+ cell pool. A large part of the lymph node T cells recirculate between blood and lymphoid tissues. In the rat, it has been estimated that only 5 per cent of the recirculating T cells are found in the blood at any time, whereas about 70 per cent are located in lymph nodes, lymph, and gut-associated lymphoid organs (8). When ^{32}P impregnated polyethylene strips are attached to intraabdominal lymphoid organs, such as the appendix or the spleen, a profound lymphopenia is induced in the animal. This does not occur when the strips are attached to the liver (11). The results of these experiments indicate that the radiation doses given to lymphoid tissues might be of greater importance than those given to peripheral blood. Furthermore, animal experiments have shown that recirculation of lymphocytes is a rapid process (7). Human peripheral lymph contains mainly T cells, and has a higher OKT4/OKT8 ratio than blood (13). We think that the rapid reduction of OKT4+ cells found in peripheral blood after intraperitoneal instillation of ^{32}P colloids may be due to irradiation of regional lymph nodes. Our observations are in accordance with those of RICHTER et coll. (21) who observed rapid and long-lasting depressions of T cell numbers and T cell responses after endolymphatic administration of ^{32}P colloids.

The prognostic significance of the results of this study remains unclear. The results should, however, be taken into account when adjuvant treatment regimens for ovarian carcinoma are planned. Clearly, clinical trials are needed to define the group of patients who will benefit from adjuvant treatment, and to define the optimum way of giving this treatment.

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REFERENCES

1. ANDERSON R. E. and WARNER N. L.: Ionizing radiation and the immune response. Adv. Immunol. 24 (1976), 215.
2. 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.
3. BOYE E., LINDEGAARD M., PAUS E., SKRETTING A., DAVY M. and JACOBSEN E.: Whole-body distribution of radioactivity after intraperitoneal administration of ^{32}P colloids. Brit. J. Radiol. 57 (1984), 395.
4. BØYUM A.: Isolation of lymphocytes, granulocytes and macrophages. Scand. J. Immunol. (1976) Suppl. No. 5, p. 9.
5. DAVY M.: The role of isotopes in the treatment of ovarian cancer. *In:* Carcinoma of the ovary, p. 243. Edited by E. Grundman. Gustav Fischer Verlag, Stuttgart 1983.
6. — STENWIG A. E. and KJØRSTAD K. E.: Early stage ovarian cancer. The effect of adjuvant treatment with a single alkylating agent. Acta Obstet. Gynecol. Scand. (in press).
7. ENGESET A.: Local irradiation of lymph nodes in rats. Morphological and functional alterations with relation to cancer therapy. Progr. Exp. Tumor Res. 8 (1966), 225.
8. FORD W. L.: Lymphocyte migration and immune responses. Prog. Allergy 19 (1975), 59.
9. GERBER M., DUBOIS J. B. and SERROU B.: Effect of low doses of irradiation on the T cell mediated cytotoxic response. *In:* Immunopharmacologic effects of radiation therapy, p. 53. Edited by J. B. Dubois, B. Serrou and C. Rosenfeld. Raven Press, New York 1981.

10. 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.
11. HOLLINGSWORTH J. W., CARR J. and FORD W. L.: Lymphopenia produced by polyethylene-^{32}P strips applied to the rabbit appendix. Cell. Immunol. 4 (1972), 407.
12. KAPLAN G. and GAUDERNACK G.: In vitro differentition of human monocytes. J. Exp. Med. 156 (1982), 1101.
13. OLSZEWSKI W. L., GRZELAK I. and ENGESET A.: Cells in lymph draining normal human skin. Monoclonal antibody analysis. Lymphology 15 (1982), 168.
14. 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.
15. — and KVALØY S.: Influence of progestogen therapy on T lymphocyte subsets. Acta Path. Microbiol. Immunol. Scand. 92 (1984), 89.
16. — BOSNES V. and GRAHM I.: Cis-platinum as adjunctive to surgery in early stage ovarian carcinoma. Effect on lymphoid cell distribution. Gynecol. Oncol. (in press).
17. PETRINI B., WASSERMAN J., BLOMGREN H. and ROTSTEIN S.: T helper/suppressor ratios in chemotherapy and radiotherapy. Clin. exp. Immunol. 53 (1983), 255.
18. PIVER M. S., BARLOW J. J. and LELE S. B.: Incidence of subclinical metastases in stage I and II ovarian carcinoma. Obstet. Gynecol. 52 (1978), 100.
19. 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.
20. 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.
21. RICHTER G., HEIDENBLUTH I. and HEIDELBACH U.: On endolymphatic radionuclide therapy of malignant melanoma and its immunological side effects. Dermatol. Montasschr. 168 (1982), 797.
22. 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. Lancet I (1972), 1352.