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THE EFFECT OF LOCAL RADIOTHERAPY ON T LYMPHOCYTE SUBSETS OF CANCER PATIENT

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In the study, we utilized the OKT monoclonal anti-human T lymphocyte antibodies by indirect immunoenzyme staining method to determine T lymphocyte subsets in the peripheral blood of 72 cancer patients treated by local radiotherapy. The haematological assays were performed immediately pre- and postradiotherapy. In the post-operative radiotherapy group (PR group) of 40 breast cancer patients treated by prophylactic postoperative radiotherapy, after radiotherapy blood platelet, white blood cell and lymphocyte count decreased significantly. CD_2, CD_4 and CD_8 cell counts all very significantly decreased. Proportion of CD_2 and CD_4 had no apparent change but proportion of CD_8 increased significantly. The group appeared apparent hypoimmunity. While the complete response group (CR group) of 32 cancer patients treated with local radiotherapy, after radiotherapy blood platelet, white blood cell and lymphocyte count had no significant decrease. CD_2, CD_4 and CD_8 cell count had no significant decrease. Proportion of CD_2, CD_4 and CD_8 had no apparent change. The CR group had no apparent hypoimmunity. Comparing decrease rate of immunologic parameters in these two groups, decrease rate of white blood cell, lymphocyte and T lymphocyte subsets count in CR group significantly were slightened rather than pR group. This study proved that effect of radiation-damaging tumor cell enhance antigenicity of the tumor and stimulate host cell immunity including a type of T-lymphogenesis. Induced hypoimmunity by postoperative radiotherapy should be avoided in this point of view.

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Key words: Antigenicity, Radiotherapy, T lymphocyte subset.

Cancer is a multistep process resulting from the accumulation of genetic events affecting cell proliferation and differentiation, involving oncogenes and tumor suppressor genes. Recent progress in cellular immunology and in molecular oncology has illuminated several new paths for searching for tumor-specific antigens capable of stimulating cellular immunity.[1,2] At present, some studies are using the contranfection method with *ras* and *myc* oncogenes to study the effect of ionizing radiation in oncogene activation, and to evaluate the possible role of radiation in multistep oncogenesis. The biological response of cells to ionizing radiation also has a genetic basis. DNA is considered to be the critical target for radiation-induced damage in a cell. Disruption in the integrity and the function of the genetic proteins contributes to cell death:[2] some studies showed that X-ray irradiation caused remarkable increases in the expression of tumor-associated antigenes (YH-206, CEA) and C-*erb*B-2 protein by flow cytometry, it is possible that enhancement of the expression of tumor-associated antigenes and C-*erb*B-2 protein, together with the enhancement of that of MHC-class I and ICAM-1, would help cytotoxic killer cells recognize the tumor cell.[3] Radiation-damaged cancer cells provide the antigenic stimulus which enhances the specific host immune reaction. However, the critical factor in this reaction is the antigenicity of the tumor. On the other hand, radiation-damaged cancer cells show

enhanced immunogenety that may trigger the general stimulation of the host cell-mediated immunity.

Those oncogene products encoding cell surface molecules to be recognized by the patient's T-cells. some studies determined that the infiltration of T-cells and their subsets increased significantly during radiotherapy than before radiotherapy and that T helper (CD_4, Th) cell subset is a major group of tumor-infiltrating lymphocytes after radiotherapy. These results suggest that they may be related to enhancement of host cell immune response caused by tumor-specific antigens.[4, 5] This author may define the effect of radiation-damaged tumor cell enhance antigenicity of the tumor as positive to host immunity. In the course of radiotherapy not only tumor cells but a part of immunocytes are killed. Lymphocytes are the most radiosensitive blood cell; their D_o is about 200 rads. Lymphocytes in the blood vessels, interstitial tissues and lymph nodes of radiotherapeutic areas are destructed by radiation unavoidably. Either number, or immune reaction against tumor of lymphocytes are decreased by radiotherapy especially prophylactic postoperative radiotherapy for breast cancer patients.[6] This author may define the effect of radiotherapy inducing lymphopenia and immunosuppress as negative to host immunity.

In the recent advances in immunology, availability of monoclonal marks for the various lymphocyte subpopulations is a new advantage to analyze the effects of radiotherapy on immunity of cancer patients. Thymus-dependent T lymphocytes are of three main types: cytotoxic T lymphocytes (CTL), T helper cells (CD_4, Th) and T suppressor cells (CD_8, Ts). Both Th and Ts cells act respectively as positive and negative regulators of immunity. Th cells stimulate antibody production and Ts cells inhibit antibody production through a process of active suppression. Th cell may play an important role in host defence against tumor cells. Some studies proved that Th decrease, Ts increase and Th/Ts ratio decrease in peripheral blood reflect development of stage, recurrence and metastasis of cancer patients. Clinically, Th/Ts subsets change may be used as a sensitive criterion to reflect host immunity against tumor.[7–9] In the study, in order to analyze the effect of local radiotherapy on immunity of cancer patients, we utilized the OKT monoclonal antihuman T lymphocyte antibodies by indirect immunoenzyme staining methods to determine change of T lymphocyte subsets in peripheral blood of cancer patients treated by local irradiation.

MATERIALS AND METHODS

From 1991 to 1992, 72 cases of cancer patients were admitted to Beijing Institute for Cancer Research for local radiotherapy. Among them 40 cases were treated previously by radical or modified radical mastectomy followed by prophylactic postoperative radiotherapy, 45 Gy in 5 weeks, was given to the axilla, supraclavicular and parasternal regions using ^{60}Co gammaray or 12–16 Mev electrons and radiotherapeutic area 200 cm. Before radiotherapy the 40 cases were not bearing tumor as control group. The characteristics of the postoperative radiotherapy group (PR group) were showed in Table 1. Other group of 32 cases of cancer patients, mean age 53 years old, man 19 and female 13, consist of breast cancer 4 cases, malignant lymphoma 11 cases, nasopharanx carcinoma 6 cases, esophagus cancer 5 cases, lung cancer 2 cases and other 4 cases, all treated by radical radiation dose and mean irradiation area was 220 cm. After radiotherapy the group had complete response according to the International Union Against Cancer (UICC) criterial for tumor response defining complete response as disappearance of all lesions for at least 4 week. The haematological assays were performed immediately pre- and postradiotherapy included peripheral white blood cell (WBC) and differential counts and T lymphocyte subsets were identified by indirect immunoenzyme staining method using CD_2 (total T cell), CD_4 and CD_8 mouse antihuman monoclonal antibody reagents. Comparison of immune parameters pre- and postradiotherapy was made with the paired *t*-test.

RESULTS

The change of pre- and post-radiotherapy im-

Table 1. Characteristics of postoperative radiotherapy group

Parameters	PR group*
No. of patients	40
Mean age	48
Clinical stage	
I	0 (0%)
II	24 (60%)
III	16 (40%)
Histological classification	
Carcinoma simplex	21 (52%)
Infiltration-ductal carcinoma	13 (33%)
Other	6 (15%)
Metastatic axillary node	
0	14 (35%)
1–3	16 (40%)
>3	10 (25%)
Site of radiotherapy	
Supraclavicular, axillary and parasternal	37 (93%)
Supraclavicular and axillary	2 (5%)
Other	1 (2%)
Average RT area	220 (cm)
Dose of radiotherapy	45–50 GY

*Postoperative radiotherapy group as the control.

mune parameters of PR group are presented in Table 2. In the group, radiotherapy was accompanied by a significant decrease in blood platelet (PLT), white blood cell (WBC), Lymphocyte (L) and CD_2, CD_4 and CD_8 counts. The total number of L decreased from the preradiotherapeutic count of 1.7±0.7 to 0.9±0.4 (10^9/L), significant at $P<0.05$. The CD_2 cells decreased significantly from 0.9±0.5 to 0.4±0.2. The CD_4 cell decreased significantly from 0.6±0.3 to 0.3±0.2. The CD_8 cells decreased significantly from 0.4±0.2 to 0.2±0.1. After radiotherapy numbers of L, CD_2, CD_4 and CD_8 cells were sharply lower than normal region. In the group, proportion of CD_2 and CD_4 had no apparent change but proportion of CD_8 increased significantly. Those results reflected immunosuppress after radiotherapy in PR group. The change of pre- and postradiotherapy immune parameters of CR group are presented in Table 3. Comparing immune parameters of preradiotherapy, PLT, WBC, L and CD_2, CD_4 and CD_8 counts of CR group all lower than PR group, in spite of this, after radiotherapy, CR group had complete response as disappearance of local lessons for at least 4 weeks, PLT, WBC, L all had no significant decrease, CD_2, CD_4 and CD_8 counts had no significant decrease, porpulation of CD_2, CD_4 and CD_8 had no apparent change. The CR group had no apparent hypoimmunity.

Table 2. Values of immune parameters in breast cancer before and after postoperative radiotherapy (n=40)

Parameters	Normal value	Pre-RT ($\bar{x}\pm s$)	Post-RT ($\bar{x}\pm s$)	*P* value*
Hb (g/L)	M. 120–150 F. 100–130	125± 16	121± 13	>0. 05
PLT (10^9/L)	100–300	164± 51	125± 32	<0.005
WBC (10^9/L)	4–10	6.9± 2	5.0±1.6	<0.005
L (10^9/L)	1–2.5	1.7± 0.7	0.9± 0.4	<0.005
CD_2 rate (%)	55–75	51± 9	54±7	>0.05
CD_4 rate (%)	35–60	32± 8	35±6	>0.05
CD_8 rate (%)	20–30	25± 5	27±4	<0.05
CD_4/CD_8	1.4–3.0	1.3± 0.3	1.3± 0.2	>0.05
CD_2 count (10^9/L)	0.5–1.9	0.9± 0.5	0.4± 0.2	<0.005
CD_4 count (10^9/L)	4.0–1.5	0.6± 0.3	0.3± 0.2	<0.005
CD_8 count (10^9/L)	0.2–0.8	0.4± 0.2	0.2± 0.1	<0.005

* The values were tested for significant difference between post and pre-radiotherapy

DISCUSSION

In present study, in PR group, the total number of L, CD_2, CD_4 and CD_8 showed a significant decrease, obviously due to radiotherapy for the breast cancer patients. Many lymphocytes in the blood circulating through the radiotherapeutic areas are killed by radiation. Destruction of lymphocytes by radiotherapy has been stated to decrease host resistance against cancer. Negative effect to immunity of radiation must be paid attention. This author used bovine thymus peptide during postoperative radiotherapy of breast cancer patients, results proved that thymus peptide enhanced a type of T-lymphogenesis, especially Th cells, suggesting protection was induced during the radiotherapy by the treatment with bovine thymus peptide.

Table 3. Values of immune parameters in CR group (n=32)

Parameter	Normal value	Pre-RT ($\bar{x}\pm s$)	Post-RT ($\bar{x}\pm s$)	*P* value*
Hb (g/L)	M. 120–150 F. 100–130	131±14	126±17	>0.05
PLT (10^9/L)	100–300	156±37	146±55	>0.05
WBC (10^9/L)	4–10	5.6±1.8	4.9±1.2	>0.05
L (10^9/L)	1–2.5	1.2±0.6	0.9±0.4	>0.05
CD_2 rate (%)	55–75	53±9	56±9	>0.05
CD_4 rate (%)	35–60	35±8	36±6	>0.05
CD_8 rate (%)	20–30	27±6	27±6	>0.05
CD_4/CD_8	1.4–3.0	1.4±0.3	1.4±0.2	>0.05
CD_2 count (10^9/L)	0.5–1.9	0.6±0.3	0.5±0.3	>0.05
CD_4 count (10^9/L)	0.4–1.5	0.4±0.3	0.3±0.1	>0.05
CD_8 count (10^9/L)	0.2–0.8	0.3±0.2	0.3±0.1	>0.05

*The values were tested for significantly different for post and pre-radiotherapy.

Table 4. Comparison of decrease rates for immune parameters in CR group and PR group

Parameters	CR group	Postsurgical group ($\bar{x}\pm s$)	*P* value*
WBC	5± 3	24± 8	<0.05
L (%)	-3± 1	40± 5	<0.05
CD_2 rate (%)	-8± 2	-5± 1	>0.05
CD_4 rate (%)	-7± 2	-10± 2	>0.05
CD_8 rate (%)	5± 1	-13± 3	<0.005
CD_4/CD_8 (%)	-8± 2	-2± 1	>0.05
CD_2 count (%)	-2± 0	39± 4	<0.05
CD_4 count (%)	4± 1	38± 4	<0.05
CD_8 count (%)	1± 0	35± 5	<0.05
PLT (%)	5± 2	18± 5	>0.05

Decrease rate=(pre-RT-post-RT)/(pre-RT)

*The value were tested for significant difference for decrease rates of CR group and PR group.

On the other hand, this study also provide that radiation-damaged tumor cell showed enhanced effect on immunity of cancer patients especially as radiotheraputic complete response group. In the CR group, in spite of that before radiotherapy immune parameters of CR group were lower than PR group, while after radiotherapy decrease of WBC, L and CD_2, CD_4 and CD_8 cells was so slight that had no significant change. As shown in Table 4, decrease rate for number of WBC, L and CD_2, CD_4 and CD_8 cells in CR group was significant smaller than PR group ($P<0.05$). This determined that there is course of supplement of regeneration immunocyte during radiotherapy in CR group accompanying dis-appearance of tumor. These results are in agreement with some studies[4,5] determined that radiation-damaging tumor cell can enhance antigenicity of tumor showing remarkable increases of expression of oncogene products. Radiation-inducing enhancement of immunogenety may trigger stimulation of host cell-mediated immunity. This study determined that radiotherapy act respectively as positive and negative effect on immunity of cancer patients. So in clinical, in order to relieve negative effect of radiotherapy on immunity of cancer patient, we must be decrease radiotherapeutic area and does for post-operative radiotherapy and treat with BRM for example of bovine thymus peptide. On the other hand, we must to increase effect of radiation-killing tumor cell inducing antigenic stimulus which caused the specific host immune reaction. Complete response and increase immunity are benefit to good prognosis for cancer patients.

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