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Effect of selected splenic irradiation on growth of meth A-fibrosarcoma in mice and partial characterization of splenic effector and suppressor cell populations.

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Abstract

Meth A-fibrosarcoma bearing BALB/c mice were subjected to selected splenic irradiation (2.0-4.0 Gy) on days 7 and 14 of tumor growth. Tumor growth was recorded by serial measurement. Irradiation given on day 7 caused regression of tumor, but irradiation given on day 14 did not show tumor regression. Antitumor activity in the Winn assay was detected in spleen cells 3 days after irradiation, but was not detected 7 days after. The cell surface phenotypes were analyzed on days 3, 7 and 14 of splenic irradiation using monoclonal antibodies (anti-Thy1.2 antibody, anti-Lyt1 antibody, anti-Lyt2 antibody, anti-L3T4 antibody) by flow cytometry. Thy 1.2, Lyt1, and L3T4 cells were increased on day 3 of splenic irradiation, but were not on days 7 and 14. Lyt2-cells did not show increase on days 3, 7 and 14. It was possibly suggested that selected splenic irradiation induced tumor regression was caused by the ability of irradiation to preferentially eliminate suppressor T cells, thereby allowing effector T-cells to become relatively dominant.

KEYWORDS: selected splenic irradiation, suppressor T-cell, flow cytometry

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Effect of Selected Splenic Irradiation on Growth of Meth A-Fibrosarcoma in Mice and Partial Characterization of Splenic Effector and Suppressor Cell Populations

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Meth A-fibrosarcoma bearing BALB/c mice were subjected to selected splenic irradiation (2.0-4.0 Gy) on days 7 and 14 of tumor growth. Tumor growth was recorded by serial measurement. Irradiation given on day 7 caused regression of tumor, but irradiation given on day 14 did not show tumor regression. Antitumor activity in the Winn assay was detected in spleen cells 3 days after irradiation, but was not detected 7 days after. The cell surface phenotypes were analyzed on days 3, 7 and 14 of splenic irradiation using monoclonal antibodies (anti-Thy1.2 antibody, anti-Lyt1 antibody, anti-Lyt2 antibody, anti-L3T4 antibody) by flow cytometry. Thy 1.2, Lyt1, and L3T4 cells were increased on day 3 of splenic irradiation, but were not on days 7 and 14. Lyt2-cells did not show increase on days 3, 7 and 14. It was possiblly suggested that selected splenic irradiation induced tumor regression was caused by the ability of irradiation to preferentially eliminate suppressor T cells, thereby allowing effector T-cells to become relatively dominant.

Key words: **selected splenic irradiation, suppressor T-cell, flow cytometry**

Hellström *et al.* (1) showed that palpable syngeneic tumors in mice were resulted in partial or complete regression by sublethal whole body x-ray irradiation. They suggested that irradiation-induced tumor regression is immunologically mediated, and that it depends on the ability of x-ray irradiation to selectively eliminate suppressor T-cells. This is one of the few convincing examples of successful immunotherapy of established tumors, but it remains to be proved that irradiation-induced tumor regression is due to an increased production of effector T-cells associated with an inhibition of suppressor T cell production.

Meyer *et al.* (2) reported that, in the syngeneic model, preliminary splenectomy delays the generation of suppressor cells. After this initial delay, with tumors having grown quite large, suppressor activity was prominent in both asplenic and intact hosts. Nonetheless, survival of asplenic animals was significantly prolonged. The spleen has been suggested as the source of suppressor cells, although suppressor cells are demonstrated in lymphnodes and thymus, as well as in peripheral blood (3, 4). Many studies have noted progressive splenic enlargement and thymic atrophy as tumors grow. A recent study found that concomitant with the generation of suppres-

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sor cells in tumor-bearing mice, aggregations of macrophages with lymphocytes in strong physical association could be identified in the spleen. Fractionation of spleen cell suspensions permitted isolation of suppressor activity to the aggregated cells (5, 6).

The purpose of this paper is to show that selected splenic irradiation, so called irradiative splenectomy, causes regression of immunogenic tumors growing in immunocompetent mice, and that tumor regression is associated with the inactivation of suppressor T-cells, resulting in the augmentation of effector T-cells.

Materials and Methods

Animals. Male BALB/c mice (6–8 weeks of age) were used in all experiments, which were purchased from Shizuoka Experimental Animal Farm Co., Ltd. (Japan).

Tumor. The methylcholanthrene-induced Meth-A fibrosarcoma, which was syngeneic for BALB/c mice, were passaged as ascites, harvested, cryopreserved, and prepared for implantation (7, 8). For experiments, tumors were initiated intradermally in the region of dorsal back by injection of 5×10^5 tumor cells in a volume of 0.1 ml of PBS. Tumor growth and regression were monitored by measuring changes against time in the mean of two diameters measured at right angles.

Irradiation. Mice were irradiated in a Toshiba, KXC-18 irradiator (165 KV, 20 mA, a 0.5 mm copper and a 0.5 mm aluminium filter, target distance at 50 cm) that delivered a midphantom dose rate of 52.0 cGy/min, and the total dose was 2.0–4.0 Gy. Before irradiation, the spleen was translocated from abdominal cavity to the subcutaneous pocket in the region of dorsal back. In order to irradiate spleens selectively, mice were entirely covered by a 5 mm thick lead board exposing only the splenic translocation region. The selected splenic irradiation was performed on day 7 and day 14 after tumor inoculation.

Winn assay. Spleen cells from day 10 of tumor growing mice were mixed with Meth-A cells and inoculated intradermally in a volume of 0.1 ml PBS at a final dose of 5×10^7 spleen cells: 5×10^5 tumor cells/mouse (9). Meth-A cells were harvested from short term tissue cultures. Spleen cells from age and sex-matched normal mice were assayed concurrently. As an additional controls mice were also inoculated with 5×10^5 Meth-A cells only. Each experimental group consisted of 10 mice.

Flow cytometric analysis of T cell surface phenotypes. To analyze the cell surface phenotypes of spleen cells, the following monoclonal antibodies were used; anti-Thy1.2-FITC, anti-Lyt1-FITC, anti-Lyt2-FITC, and phycoerythrin-avidin conjugated L3T4, which were purchased from Becton Dickinson Monoclonal Antibody Center Co., Ltd., CA, USA. Measurements were performed by FACS III (Becton Dickinson FACS Systems) on days 3, 7 and 14 after irradiation (10).

Results

Effects of selected splenic irradiation on tumor growth. Meth-A tumor bearing mice were subjected to selected splenic irradiation (2.0

Table 1 Restoration of anti-tumor activity after splenic irradiation in Winn assay

Cells	Irradiation (Gy)	Winn assay recipients[d]	
		Tumor incidence	Mean tumor volume (±SD)(mm^3)
Tumor cell only	—	10/10	3990±665
Tumor bearer[a]	0	10/10	3838±458
Tumor bearer[b]	4.0	6/10	479±289[e]
Tumor bearer[c]	4.0	10/10	3520±857

a: Spleen cells were sampled on day 10 after tumor inoculation.
b: Tumor bearers were irradiated on day 7 after tumor inoculation.
c: Tumor bearers were irradiated on day 7 after tumor inoculation. Spleen cells were sampled 7 days later.
d: Mice were 10 in the group.
e: $p<0.01$.

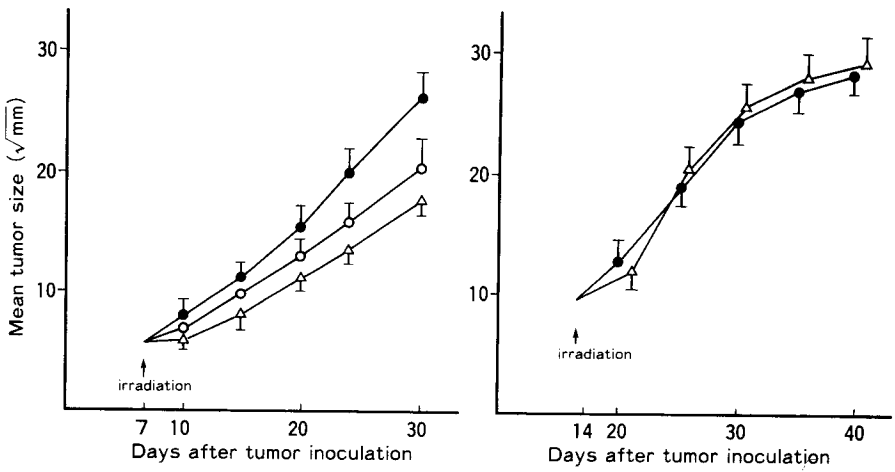

Fig. 1 Anti-tumor effect of splenic irradiation. Nonirradiated control is indicated by the closed circle (●). 2.0 Gy(○) or 4.0 Gy (△) splenic irradiation of mice bearing a day 7 tumor, caused partial tumor regression (left panel). It did not have effect on the mice bearing a day 14 tumor (right panel). Means (± SD) of ten mice per group.

-4.0 Gy) on days 7 and 14 of tumor inoculation. Fig. 1 showed evidence that 2.0 or 4.0 Gy of splenic irradiation of mice bearing a day 7 tumor caused tumor regression, but that irradiation given on day 14 did not show tumor regression.

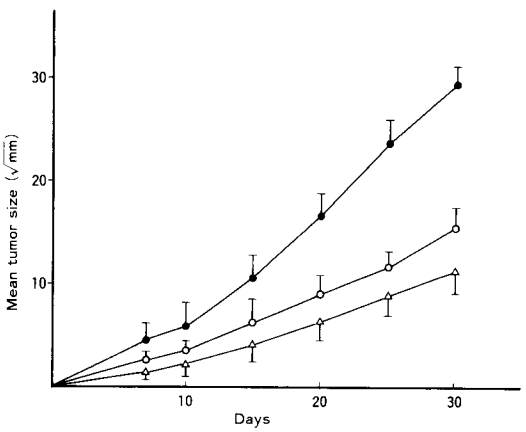

Fig. 2 Anti-tumor immunity after various splenic irradiation. Normal mice were implanted with 5×10^5 tumor cells alone (●, control). Tumor growth was inhibted by the mixture of irradiated spleen cells. Spleen cells were sampled 3 days after 2.0 Gy (○), or 4.0 Gy (△) irradiation. Means (± SD) of ten mice per group.

These results indicate that the therapeutic effect of splenic irradiation depends on the stage of the tumor at irradiation.

Anti-Meth-A activity of spleen cells of splenic irradiated and tumor bearing mice. Anti-Meth-A activity in the Winn assay was analyzed in spleen cells of the following groups: (a) day-10 tumor bearing mice, (b) day-10 tumor bearing mice with splenic irradiation on day 7, (c) day 14 tumor bearing mice with splenic irradiation on day-7. Antitumor activity was detected in Group (b), but was not detected in Groups (a) and (c) (Fig. 2, Table 1). These results suggest that the antitumor effect of splenic irradiation decreased gradually after irradiation.

Flow cytometric analysis of spleen cells surface phenotypes. The cell surface phenotypes of spleen cells were analyzed on days 3, 7 and 14 after splenic irradiation. Thy1.2, Lyt1 and L3T4-cells were increased on day 3 of splenic irradiation, but were not on days 7 and 14. Lyt 2 cells did not show increase on days-3, 7 and 14 (Fig. 3).

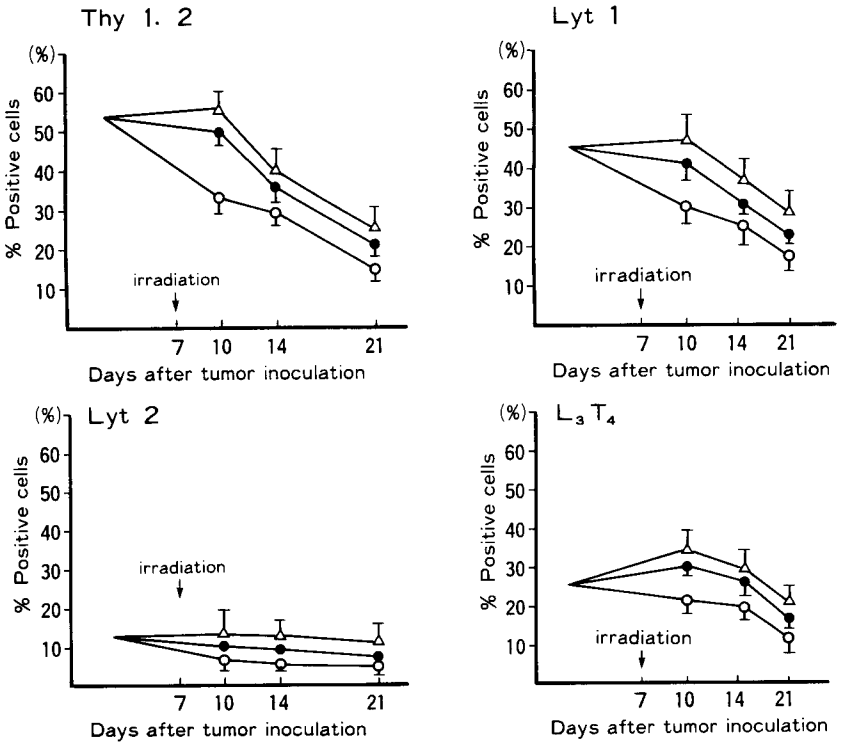

Fig. 3 Immunofluorescence analysis of the spleen cells after splenic irradiation. Thy1. 2, Lyt1, Lyt2, and L3T4 positive spleen cells were reduced in possibility with tumor growth (●). Splenic irradiation caused their increase of possibility statistically with the exception of Lyt2 cells. Means (± SD) of ten mice per group. (○; 2.0 Gy, △; 4.0 Gy)

Discussion

Immunological antitumor resistance directed to syngeneic or autologous tumor can be induced in either tumor bearing hosts or hosts which have been immunized with homologous attenuated tumor cells. It was found that the cytotoxic T-lymphocytes (CTL) directed to syngeneic or autologous tumor cells comprise one of the major effector cells with antitumor resistance in the mouse (7, 8, 11). On the other hand, Fujimoto *et al.* reported that suppressor T-cells capable of specifically inhibiting the induction and activation of CTL, are strongly activated in tumor-growing hosts, thus indicating that the suppressor T-cell regulates the CTL response to either syngeneic or autologous tumors. On the basis of these findings, it is suggested that T-cell response to syngeneic or autochthonous tumor is directly involved in both regression and growth of tumors, and that tumor immunity depends on a balance between the activation of the CTL and that of the suppressor T-cell directed to the homologous tumor in the mouse (12–15).

The sensitivity of suppressor T-cells or their precursors to irradiation has been well documented and it is known that the suppressor T-cells in general are more radiosensitive than other lymphocytes (16–20). Suppressor T-cells are demonstrated in lymphnodes and thymus, as well as in peripheral blood. However, the spleen is practically the source of the suppressor T-cells (3, 4). This is the reason why we devised the selected splenic irradiation.

Our results serve to confirm the findings by other authors that a palpable immunogenic tumor in mice undergoes partial regression by sublethal dose of ionizing radiation and depends on its

immunogenicity (21). Irradiation given on day 7 caused regression of tumor, and antitumor activity in the Winn assay was detected in spleen cells 3 days after irradiation. Thy1.2$^+$-cells, Lyt1$^+$, L3T4$^+$-cells were increased on day 3 after splenic irradiation, but Lyt2 cells were not increased. This result suggests that splenic-irradiation-induced tumor regression is associated with the failure of the host to generate suppressor T-cells and augmented production of effector T-cells.

It is interesting that exposure to splenic irradiation on day 14 of tumor growth does not result in the onset of tumor regression. Unlike most other mammalian cells that are radioresistant in a resting state and radiosensitive in cell-cycling, it is apparent that lymphocytes are radiosensitive when resting and radioresistant while they are in cell-cycling (22). Our present research suggested that spleen cells on day 7 of tumor growth were resting, and spleen cells on day 14 were cycling. This might be one of the reasons why irradiation given on day 7 caused tumor regression, but that irradiation given on day 14 failed to induce regression. In other words, a given time is required for successful immunotherapy of selected splenic irradiation.

In order to establish a rational, specific cancer immunotherapy, we have to devise a way not only to augment specific CTL response, but also to inactivate the suppressor T-cell response against autochthonous tumor. We succeeded in inactivating the suppressor T-cell response, resulting in the augmentation of CTL response, by selected splenic irradiation. The present research, however, elucidated that the immunoaugmenting effect of irradiation was not sustained. Thy1.2, Lyt1, and L3T4-cells were increased on day 3 after splenic irradiation, but were not on day 7 or 14. The splenic irradiation, therefore, should be repeated for successful immunotherapy, so that the host is unable to generate suppressor T-cells with the ability of sustained production of effector cells.

Development of suppressor cell activity and loss of protection may be done by manipulation of cellular immune response with selected splenic irradiation. Further research should be done to detect the physiological difference between activated effector T-cells and inactivated suppressor T-cells for more effective and acceptable cancer therapy.

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