Plaque-Forming Spleen Cells Following Irradiation of X5563 Tumor* (35598)

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Previous studies have shown that plaque-forming cell counts in the spleens of C_3H mice are significantly reduced in the presence of solid plasma-cell tumors weighing 10–12% of the body weight of the host mouse (1, 2). Circulating antibody titers in such mice average below the titers of their nontumorous controls after a single immunization with sheep erythrocytes. Other studies had shown that restoration of the hemolysin titers after a primary immunization could be brought about by treatment of the solid tumor with radiation (3, 4) or by its surgical removal (5).

Experimental results described in this report show that after radiation-induced regression of the solid tumor both plaque-forming cell counts and circulating antibody titers are restored to control values in mice singly immunized with sheep erythrocytes. Moreover, these beneficial effects on the immune response were observed in mice immunized immediately after radiation exposure of the tumor as well as in mice whose tumors had totally regressed following radiation therapy.

Materials and Methods. C_3H/eB mice of both sexes, 10–15 weeks of age, received a subcutaneous inoculation in the right hind flank of 0.03 ml of a freshly prepared tumor cell suspension in physiological saline. The tumor, X5563,[1] was in its transfer generations 127 to 133 during the course of the experiments reported below. Successful transplants became palpable usually within 1 week after transfer and periodic external measurements with calipers were made to follow the tumor growth to the point of subsequent experimental treatment. Untreated tumors generally were fatal to the mice by 18 days after transplant.

At appropriate times, depending on the specific treatment schedule, the tumorous mice were given a single intraperitoneal injection of a suspension of sheep erythrocytes (SRBC) containing approximately 9×10^8 cells. Tumorous mice and their corresponding controls were sacrificed for their spleens and serums on the fifth day after immunization. Hemolysin and hemagglutinin titers were estimated by microtitration procedures using 2-fold dilutions. The highest serum dilution yielding complete hemolysis or unequivocal agglutination was recorded as the titer for each serum sample. Plaque-forming cell counts (PFC) were made as described previously (1) and were recorded as PFC/10^6 viable spleen cells.

Solid tumors in some of the treatments described here were exposed to 6.75 k rads of ^{137}Cs gamma rays in a small animal irradiator.[2] Radiation exposures of the tumors were carried out by placing the tumorous animal in a plastic cartridge in such a way that the tumor and overlying skin were brought through a slot to the outside of the cylinder. A sliding block and fitted closure immobilized the mouse during irradiation. The plastic cartridge and mouse were shielded by 5 cm of lead so that only the tumor would "see" the source of radiation. Radiation dose rate to the tumor was 150 rads/min at a target distance of 10.3 cm and ferrous sulfate dosimetry assured that the mouse was exposed to negligible amounts of radiation during the exposure of its tumor. In preliminary tests, using total exposure doses ranging from 1 to

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[1] Initial inoculum of this tumor was in its one hundred twenty-second transfer generation when it was kindly supplied by Dr. Michael Potter, Laboratory of Biology, National Cancer Institute, Bethesda, Md.

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11.25 k rads, it was observed that exposures greater than 6.75 k rads produced severe skin lesions subsequent to irradiation although the tumors regressed while lower exposure doses resulted in poor tumor regression rates.

Results. Groups of mice were sacrificed from 6 to 14 days after tumor inoculation and at 5 days after a single immunization with SRBC in order to observe the effect of tumor size on both PFC in the spleens and circulating antibody. Data summarized in Table I show the results of this treatment and demonstrate an increasing suppressive effect of the growing X5563 tumor on the immune response. The tumors of the mice of Table I ranged from neoplasms too small to dissect and weigh accurately to solid masses comprising nearly 17% of the body weight. Variability in the observed PFC counts and circulating titers shown by the large error terms in Table I may be partly due to the fact that some tumorous mice with very small tumors had both unusually low PFC counts and low circulating antibody titers compared to other mice with tumors of nearly the same size. This observation suggests that some of the inocula may have produced tumors that more effectively depressed the immune mechanism than was true in closely similar experimental animals. Increase in solid tumor mass accompanied a variable increase in average spleen weight as seen in Table I. Slightly higher average body weights were associated with the larger tumors probably due to the tumor mass. Occasionally the tumors metastasized, increasing the body weight by 3–4 gms before the animal died. Such animals were rejected for the purposes of the experiments reported here.

Average PFC and hemolysin titers observed for the mice of Table I were compared graphicaly to tumor size at sacrifice. The drop in hemolysin titers averaged about 1 log and roughly parallels the fall in PFC in the spleens as the tumors increased in percentage of body weight, as summarized in Fig. 1.

Irradiation of the solid tumor mass resulted in its regression in about 85% of the attempts at therapy. Figure 2 compares the change in solid tumor size in a group of 20 mice successfully responding to the radiation treatment with the tumor volume in 10 other mice whose tumor growth was only briefly interrupted by the irradiation. By 8 days after irradiation, tumors which were satisfactorily destroyed were just palpable and had completely disappeared at time of sacrifice from 13 to 28 days after irradiation. During the course of these experiments 5 mice whose tumors had been irradiated and regressed were held in the laboratory for 3 months after therapy with no evidence of the return of the neoplasm.

Results summarized in Table II show that total radiation-induced tumor regression was associated with a restoration of the immune

TABLE I. Effect of X5563 Tumor Weight on the Primary Immune Response in C_3H/eB Mice Sacrificed 5 days after Immunization with SRBC.

	Average					
				Circulating antibody titers (± SE)		
No. of mice	Body wt. (g ± SE)	Tumor wt. (g ± SE)	PFC/10^6 spleen cells (± SE)	Hemolysin	Agglutinin	Spleen wt. (g ± SE)
22	23.9 ± 0.5	0[a]	115.0 ± 10	1307 ± 319	1154 ± 227	0.16 ± 0.005
10	24.4 ± 0.4	0.29 ± 0.007	107.6 ± 16.5	1555 ± 389	2057 ± 394	0.18 ± 0.007
14	25.1 ± 0.5	1.48 ± 0.07	48.6 ± 8.3	2272 ± 437	2460 ± 506	0.21 ± 0.009
5	25.2 ± 1.0	2.46 ± 0.13	26.0 ± 12.7	976 ± 256	1208 ± 501	0.29 ± 0.004
3	25.0	3.4	17.8	669	800	0.23
14	27.4 ± 1.3	4.51 ± 0.67	8.7 ± 2.3	260 ± 71		0.18 ± 0.02

[a] Mice sacrificed within a week after tumor inoculation produced tumors too small to dissect and weigh successfully.

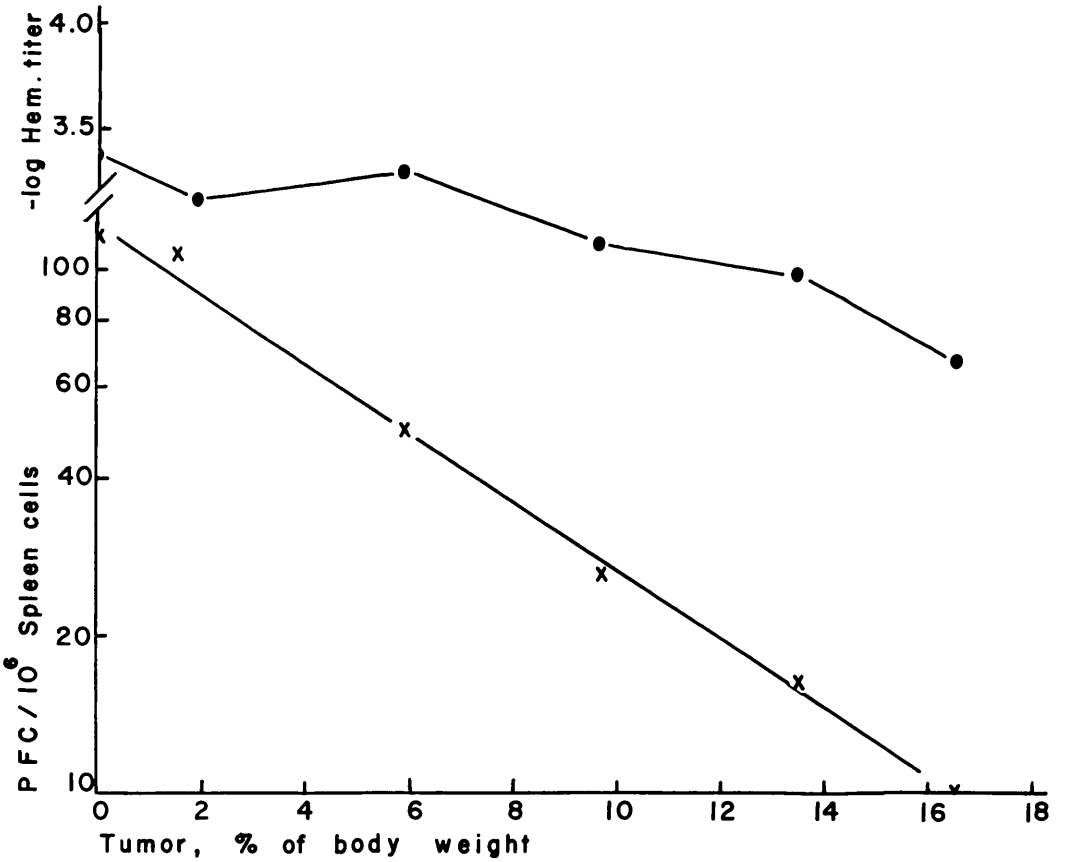

FIG. 1. Average plaque-forming cell counts (lower) and circulating hemolysin titers (upper) in C_3H/eB mice bearing X5563 tumors of the indicated size at the time of sacrifice.

response to near the values found for the nontumorous control mice. Thus, 20 mice, group R of Table II, had an average of 120 PFC/10^6 spleen cells compared to about 107 PFC in their nontumorous controls while mice bearing actively growing tumors averaging 4.5 g and 16.5% of the body weight at the time of sacrifice had an average of only about 9 PFC/10^6 spleen cells. Data from 2 mice were included in group R, Table II, in which at autopsy there was some evidence of metastasis. These 2 animals had counts of 0 and 20 PFC/10^6 spleen cells. Circulating hemolysin titers in the tumor regressed group averaged near the values for their nontumorous controls.

In a group of 14 mice immunized within 2–3 hr after tumor irradiation, group I of Table II, PFC counts averaged 485/10^6 spleen cells or more than 4 times higher than the average counts observed with spleens of the nontumor bearing controls. This observation indicates that the suppressive effect of the tumor on PFC counts was interrupted promptly after exposure to 6.75 k rads. In keeping with the improved average PFC counts the circulating hemolysin titers in the mice of group I, Table II also averaged somewhat higher than titers in the group R mice. The PFC counts ranged from a low value of 50/10^6 spleen cells to 1278 PFC in 14 mice of group I with a median count of 335 compared to a range of 0–514 and a median of 83 PFC/10^6 spleen cells in the 20 mice of group R, Table II. Average tumor weight in the mice of group I, Table II was 0.44 g reflecting the observed drop in estimated mean tumor volume from 1.74 cc at irradiation to 0.41 cc at sacrifice 5 days after immunization and exposure to radiation.

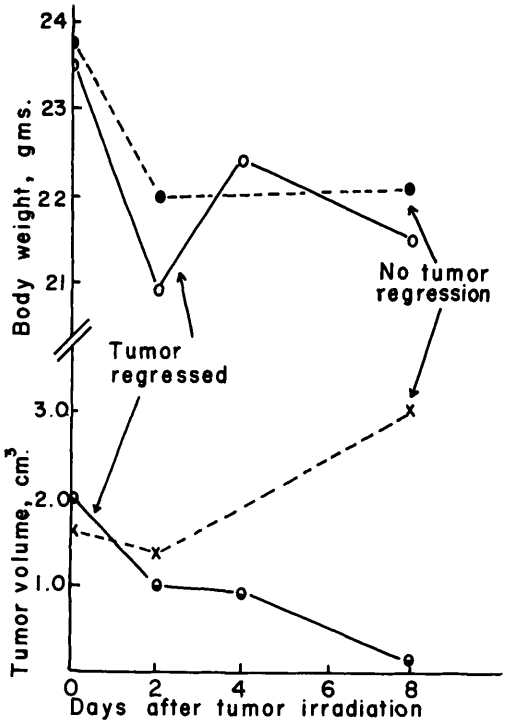

FIG. 2. Body weights and tumor volumes compared for mice whose tumors were exposed to 6.75 k rads on day 0. Average body weights in grams and tumor volumes in cm^3 for 20 successfully treated mice (—); and for 10 mice in which the tumors failed to regress, (- - -).

Discussion. Experiments are described which show that successful radiation therapy of the solid, transplanted X5563 tumor is associated with recovery of the PFC count in spleens and circulating hemolysin titers in C_3H/eB mice after a single immunization with SRBC. Recovery of the primary immune response was observed in mice after their tumors had completely regressed as a result of their exposure to 6.75 k rads of ^{137}Cs gamma radiation and an enhancement both of average PFC counts and circulating antibody in a group of mice whose tumors had been similarly irradiated up to 3 hr before primary immunization.

Such observations raise the question as to the mechanism responsible for the suppression of IgM synthesis in tumorous mice not treated with radiation. Possible competition between the neoplastic plasma cells for the proteins needed to synthesize the abnormal globulins characteristic of this tumor (6) and those needed by the antibody producing cells for the synthesis of IgM is an attractive consideration. Inhibition of IgM synthesis by the abnormal globulins produced by the neoplasm might have taken place by means of a process similar to the one outlined by Moller (7) and cited in a recent report from this Laboratory (1). On this basis, however, the prompt recovery of the primary immune response which followed the tumor irradiation requires the assumption both that the syn-

TABLE II. PFC and Circulating Antibody in C_3H/eB Mice Immunized Immediately (I) and 13–28 Days (R) after Exposure of X5563 Tumor to 6.75 K rads of ^{137}Cs Gamma Radiation.

Treatment	No. of mice	Average PFC/10^6 spleen cells (± SE)	Average Circulating hemolysin titers (± SE)	Average Tumor wt. (g ± SE)	Average Spleen wt. (g ± SE)
No tumor controls					
Nonimmune	3	0.6	0	—	0.08
Immune	14	107.2 ± 12.3	814 ± 354	—	0.11 ± 0.005
Tumorous controls					
Immune	11	8.7 ± 2.2	260 ± 71	4.5 ± 0.6	0.18 ± 0.02
Experimental					
I[a]	14	485.1 ± 98.5	970 ± 140	0.44 ± 0.04	0.18 ± 0.01
R[b]	20	120.4 ± 26.5	827 ± 231	Regressed	0.13 ± 0.008

[a] Mice of this group were immunized within 2–3 hr after tumor irradiation.

[b] Mice of this group were immunized at from 13 to 28 days after the tumor was irradiated. Tumors had an average volume of 2 cc at the time of irradiation.

thesis of the abnormal globulins ceased abruptly after tumor irradiation and that the offending circulating abnormal globulin was either quickly (within less than 5 days) reduced below a limiting amount or was somehow ineffective in suppressing IgM synthesis during the 5 days after immunization and irradiation in the mice of group I, Table II.

Numerical inhibition of cells competent to produce IgM(hemolytic antibody) as a by-product of the neoplastic growth, is untenable because of the prompt recovery and even enhancement of PFC count observed in the spleens of group I mice of Table II following radiation exposure. Moreover, the absence of extraordinary enlargement of the spleens of the tumorous mice seems to rule out the possibility that replacement of normal antibody producing cells had taken place. Experiments to be presented in a later report from this Laboratory are being carried out to determine the exact time during the induction phase of IgM production at which the suppressive action of the tumor may first be observed.

The enhancement of the average PFC counts and circulating antibody titers in the mice whose tumors were irradiated just prior to immunization suggests a possible adjuvant action resulting from the treatment. Irradiation of the tumor and its subsequent disappearance would have been accompanied by an appreciable increase in macrophage activity at the site of the injury and associated with the mass of damaged and dying cells. It is possible that tissue products from the (formerly) rapidly growing neoplastic tissue may have stimulated present and already primed normal cells to antibody production through a mechanism comparable to that reported for rat macrophages (8) and mouse peritoneal cells (9). Studies of this aspect of the response of the X5563 tumor to irradiation are in progress in this Laboratory in order to further elucidate the mode of the deleterious effect on IgM production in host mice challenged with sheep erythrocytes.

Summary. Exposure of the transplanted plasma cell tumor X5563 in C_3H/eB mice to 6.75 k rads of ^{137}Cs gamma radiation resulted in complete regression in about 85% of the treated tumors. Primary immunization of experimental mice with sheep erythrocytes done 13–28 days after tumor irradiation was associated with spleen PFC counts and hemolysin titers on the fifth day following that were within the range observed for nontumorous controls. Mice whose tumors were irradiated within 3 hr before immunization had nearly four times more PFC on the average. The way in which the X5563 tumor produces suppression of IgM synthesis is not clear although its prompt recovery following tumor irradiation suggests that the tumor's suppressive action is closely linked with the active metabolic state of the growing neoplasm.

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