

RADIATION-INDUCED, IMMUNOLOGICALLY MEDIATED
REGRESSION OF AN ESTABLISHED TUMOR AS AN
EXAMPLE OF SUCCESSFUL THERAPEUTIC
IMMUNOMANIPULATION

Preferential Elimination of Suppressor T Cells Allows Sustained
Production of Effector T Cells

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It was shown several years ago by Hellström et al. (1) that sublethal x irradiation of mice bearing palpable syngeneic tumors can result in partial or complete tumor regression. These same authors showed, in addition, that the therapeutic effect of irradiation depended on the tumors being above a certain size, and that regression failed to occur if the mice were infused, immediately after irradiation, with T cells from normal donor mice. It was logical to suggest, therefore, that irradiation-induced tumor regression is immunologically mediated, and that it depends on the ability of x irradiation to selectively eliminate suppressor T cells (1). Considering that this is one of the few convincing examples of successful immunotherapy of established tumors, I considered it important to investigate it in more detail with the tumors under study in this laboratory. There remained a need, for example, to provide causal evidence that irradiation-induced tumor regression is immunologically mediated, and to document that it is associated with increased production of effector T cells on the one hand, and with the absence of suppressor T cell production on the other.

Assays that can measure the tumor-induced production of effector and suppressor T cells have been developed in this laboratory as a result of an investigation (2, 3) of the generation and decay of concomitant immunity to several immunogenic tumors. It was revealed (4, 5) with the tumors under study in this laboratory that between days 6 and 9 of tumor growth, the tumor-bearing host generates tumor-sensitized T cells that can, on passive transfer, cause regression of a small tumor in irradiated recipients. After day 9, however, these effector T cells are progressively lost, and this is temporally associated with the progressive acquisition of T cells that can, on passive transfer, suppress the expression of adoptive immunity against an established tumor in test recipients. Therefore, during progressive tumor growth, the abridgement of effector T cell production is associated with the onset of suppressor T cell production. It was hypothesized, therefore, that immunogenic tumors grow progressively, in spite of their posses-

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sion of transplantation rejection antigens, because the immune response they evoke in their immunocompetent hosts is downregulated by suppressor T cells before enough effector T cells are produced to cause tumor regression (4, 5).

The purpose of this paper is to show that whole-body γ irradiation can cause complete regression of immunogenic tumors growing in immunocompetent, but not in immunoincompetent mice, and that regression can be inhibited by infusion of L3T4⁺ suppressor T cells from tumor-bearing donors. It will show, in addition, that tumor regression is associated with a sustained production of effector T cells, and with a failure to produce suppressor T cells.

Materials and Methods

Mice. BALB/c, DBA/2, A/Tru, CB6 (BALB/c \times C57BL/6), B6D/2 (C57BL/6 \times DBA/2), and AB6 (A/Tru \times C57BL/6) adult mice (10–12 wk of age) were obtained from the Trudeau Institute Animal Breeding Facility. The mice were reared under barrier-sustained conditions, and were shown to be free of common viral pathogens according to tests routinely performed by the Diagnostic Testing Service of Microbiological Associates, Bethesda, MD.

Tumors. The Meth A fibrosarcoma (BALB/c), P815 mastocytoma (DBA/2), L5178Y lymphoma (DBA/2), P388 lymphoma (DBA/2), and SA1 sarcoma (A/J) were passaged as ascites, harvested, cryopreserved, and prepared for implantation as described previously (4, 6). The origins of these tumors have also been described in previous publications (4–6). For experiments, tumors were initiated intradermally in the belly region of semisynthetic hosts by injection of 10^6 tumor cells in a volume of 0.05 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 ^{137}Cs irradiator (Atomic Energy of Canada Ltd., Ottawa) that delivered a midphantom dose rate of 30 rad/min.

Assay for Suppressor T Cells. The presence of suppressor T cells in the spleens of tumor bearers was determined by measuring the capacity of spleen cells to suppress, on intravenous infusion, the ability of passively transferred immune spleen cells from tumor-immune donors to cause regression of a 4-d intradermal tumor in T cell-deficient test recipients, as described in previous publications (4, 5). Recipient test mice were made T cell deficient (TXB)¹ by thymectomy at 6 wk of age, followed 1 wk later by lethal (900 rad) γ irradiation. Immediately after irradiation they were infused intravenously with 10^7 syngeneic bone marrow cells and they were used after an additional 4 wk. Donors of immune spleen cells were mice that had been immunized several weeks earlier by intradermal injection of an admixture of $1\text{--}2 \times 10^6$ living tumor cells and 100 μg of *Propionibacterium acnes* (formalin-killed *Corynebacterium parvum* from Burroughs Wellcome Co., Research Triangle Park, NC). At the time of harvesting their spleen cells, the donors possessed a state of immunological memory (7). Their spleens were diced into small pieces and pushed through a 60-mesh stainless screen into PBS. The resulting cell suspension was triturated to break up clumps, passed through sterile surgical gauze to remove debris, washed twice in PBS, and was resuspended in PBS for intravenous infusion.

Suppressor donors were mice bearing one or another of the tumors under study. Suspensions of donor spleen cells were obtained by the method used for obtaining suspensions of immune T cells. The suppressor assay involved infusing TXB recipients bearing a 4-d intradermal tumor intravenously with one spleen equivalent ($\sim 1.5 \times 10^8$) of immune spleen cells, followed 1 h later by infusion of one spleen equivalent ($1.5\text{--}2.0 \times 10^8$) of suppressor T cells from donors bearing an established tumor. With this assay, the level of suppression transferred is indicated by the degree to which immunologically mediated regression of the recipient tumor is inhibited (4, 5).

Assay for Effector T Cells. The assay for measuring the production of effector T cells

¹ Abbreviation used in this paper: TXB, T cell-deficient.

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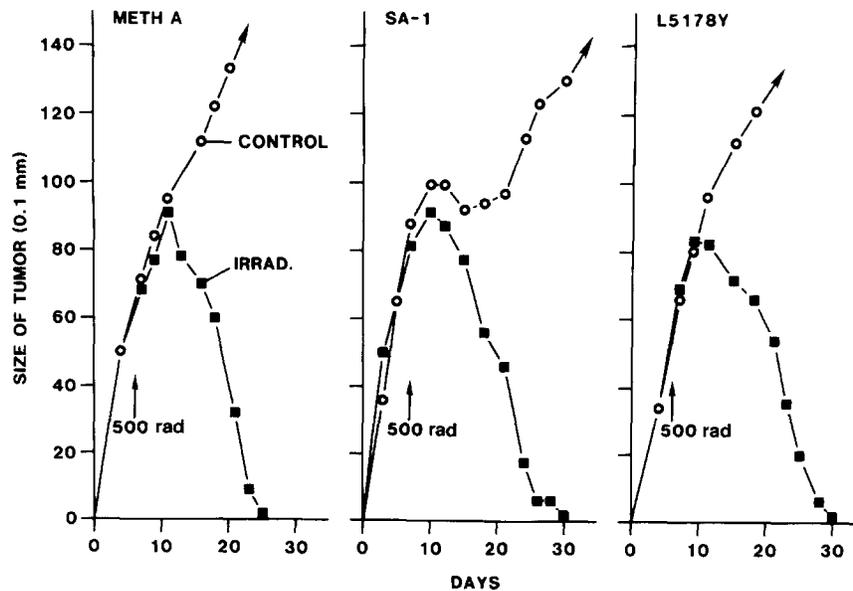


FIGURE 1. Evidence that 500 rad of whole-body γ irradiation of mice bearing a day 6 tumor (arrow) caused complete tumor regression, after a 3–4 d delay. The same result was obtained with the Meth A fibrosarcoma, SA1 sarcoma, and L5178Y lymphoma, each growing from an intradermal implant. Means of five mice per group.

in mice bearing a progressive tumor has been described previously (4, 5). It involved determining the capacity of one organ equivalent of splenic T cells from tumor bearers to cause, on passive transfer, regression of a 3-d tumor in recipients that were exposed to 500 rad of γ radiation 1 h before receiving spleen cells. The method for obtaining spleen cells was the same as described above.

Deleting T Cell Subsets. T cell subsets were deleted by treating spleen cells with culture medium in which hybridomas (5×10^5 cells/ml) secreting anti-Ly-1.2 antibody (clone CP30 from Dr. Jan Klein, Max Plank Institute, Tubingen, Federal Republic of Germany), anti-Ly-2.2 antibody (clone TIB-150 from the American Type Culture Collection, Rockville, MD), or anti-L3T4 antibody (clone GK-1.5 from Dr. Frank Fitch, Department of Pathology, University of Chicago, IL)) had been growing. The spleen cells were treated at 2×10^7 cells/ml for 30 min at 4°C in the appropriate antibody-containing culture medium, and then with the same volume of a 1:10 dilution of rabbit serum as a source of complement, as previously described (4, 5).

Results

γ Irradiation Resulted in Regression of Three of Five Tumors Tested. It was necessary first to confirm the findings of others (1) that whole-body irradiation can cause partial or complete regression of immunogenic tumors. It can be seen in Fig. 1 that 500 rad of γ radiation given on day 6 of tumor growth caused complete regression of the Meth A fibrosarcoma, SA-1 sarcoma, and L5178Y lymphoma, with the onset of regression occurring after a delay of several days.

In contrast, Fig. 2 shows that the same dose of γ radiation had only a marginal therapeutic effect against a 6-d P815 mastocytoma, and no therapeutic effect at all against a 6-d P388 lymphoma. Since all of the tumors tested are known to be immunogenic (capable of immunizing against growth of a subsequent implant),

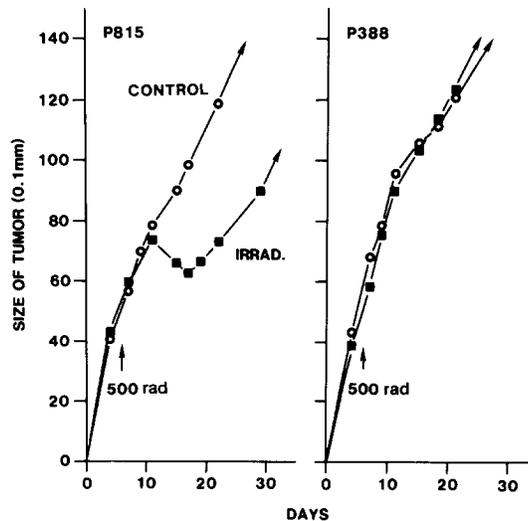


FIGURE 2. Evidence that 500 rad of whole-body γ irradiation (arrow) did not cause regression of the less immunogenic P815 mastocytoma, or P388 lymphoma. Means of five mice per group. ■, irradiated; O, control.

it is apparent that these latter two tumors are less immunogenic than the others, assuming that irradiation-induced regression is immunologically mediated.

Evidence that Irradiation-induced Tumor Regression Is Immunologically Mediated. If irradiation-induced tumor regression is immunologically mediated, it would follow that whole-body irradiation should have no effect against a tumor growing in mice that have been rendered incapable of generating an antitumor immune response. This prediction was tested by determining whether 500 rad of γ irradiation would cause regression of a 6-d Meth A fibrosarcoma growing in TXB mice. Fig. 3 clearly shows that, whereas γ irradiation caused complete regression of the Meth A tumor growing in immunocompetent mice, it had no therapeutic effect at all against the same sized tumor growing in TXB mice. Therefore, irradiation-induced tumor regression depends on an intact T cell system.

Irradiation-induced Regression Is Blocked by Infusion of $L3T4^+$ T Cells from Tumor-bearing Donors. According to the results of others (1), the therapeutic effect of irradiation can be blocked by infusing the tumor-bearing host, immediately after irradiation, with T cells from normal mice. However, attempts in this laboratory to confirm this result with the Meth A fibrosarcoma were unsuccessful, in that infusion of as many as three spleen equivalents of normal T cells failed to interfere with the therapeutic effect of irradiation. On the other hand, it proved easy to show in a routine fashion that the therapeutic effect of irradiation against the Meth A tumor could be blocked by infusing the host with spleen cells from donor mice bearing an established Meth A tumor. It can be seen in Fig. 4 that, whereas infusion of 3×10^8 spleen cells from normal donor mice failed to inhibit regression of an established Meth A tumor in γ -irradiated recipients, 1.5×10^8 spleen cells from donors bearing a 6-d or a 16-d Meth A tumor inhibited tumor regression in all mice. It needs to be pointed out, moreover, that the results of

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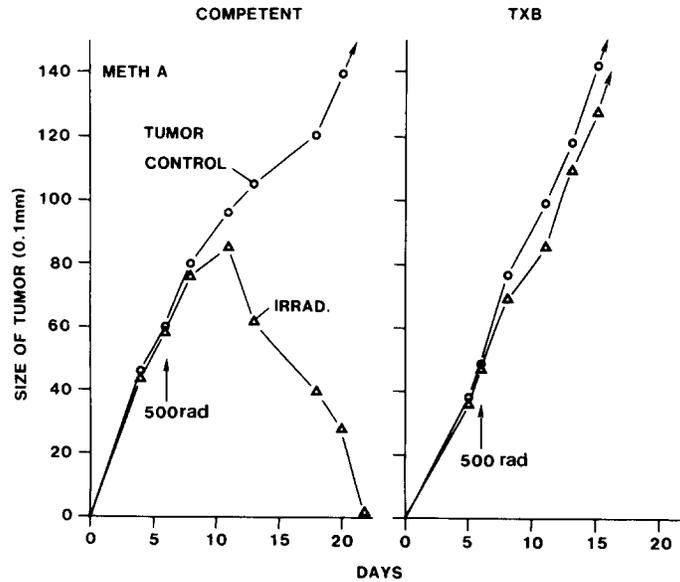


FIGURE 3. The therapeutic effect of 500 rad of γ irradiation was T cell dependent. Whereas irradiation causes complete regression of the Meth A fibrosarcoma growing in immunocompetent mice (*left panel*), it had no effect on the same-sized tumor growing in TXB mice (*right panel*). Means of five mice per group. *Irrad.*, irradiation.

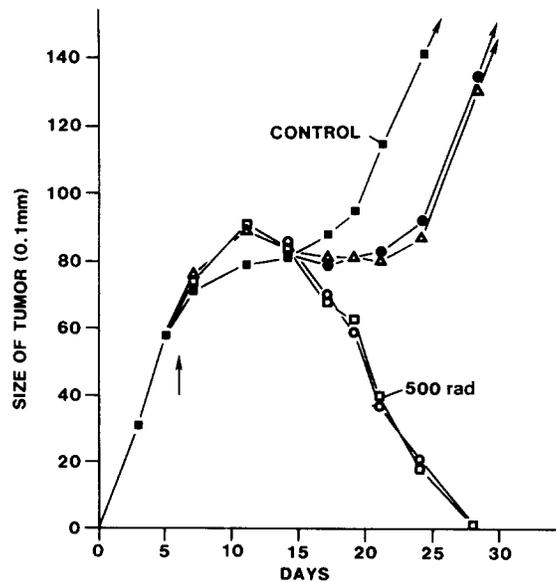


FIGURE 4. γ irradiation-induced regression of the Meth A fibrosarcoma was inhibited by infusing the tumor bearer, immediately after irradiation on day 6 (*arrow*), with one organ equivalent (1.5×10^8) of spleen cells from donors bearing a day 6 or day 16 tumor, but not by three organ equivalents (3×10^8) of spleen cells from normal donors. Means of five mice per group. ●, day 6 spleen; △, day 16 spleen; ○, normal spleen.

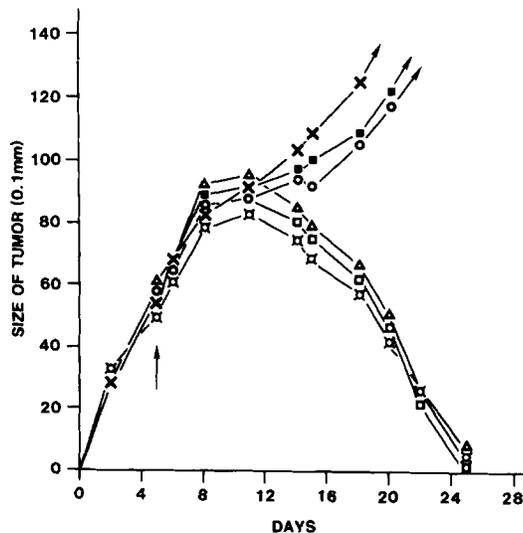


FIGURE 5. Evidence that the spleen cells from donors with day 16 tumors that, on passive transfer, inhibit irradiation-induced regression of a day 6 tumors were $\text{Ly-1}^+, 2^-, \text{L3T4}^+$ T cells. Their suppressor function was abolished by treatment with anti-Ly-1.2 antibody and complement, or with anti-L3T4 antibody and complement, but not by treatment with anti-Ly-2.2 antibody and complement. Means of five mice per group. \times , tumor control; \circ , 500 rad; \circ , 500 rad + suppressor; Δ , 500 rad + suppressor anti-Ly-1, \square , 500 rad + suppressor anti-L3T4a; \blacksquare , 500 rad + suppressor anti-Ly-2.

two additional studies (not shown) revealed that spleen cells from 16-d tumor bearers were more reliable in blocking the therapeutic effect of irradiation than spleen cells from donors bearing a day 6 tumor, in that in these additional experiments only the spleen cells from day 16 tumor bearers inhibited tumor regression in all recipients. This perhaps is not surprising, given that tumor-induced suppressor T cells of the type under study in this laboratory can be detected in increasing number after about day 9 of tumor growth (4, 5). These tumor-induced suppressor cells have been shown to be $\text{Ly-1}^+, 2^-, \text{L3T4}^+$ T cells that can, on passive transfer, suppress the expression of adoptive immunity against an established tumor in TXB test recipients (4-5).

Evidence that T cells with the same surface phenotype were responsible for inhibiting irradiation-induced regression of the Meth A fibrosarcoma is provided by the results in Fig. 5. It can be seen that the ability of spleen cells from donors with a day 16 tumor to inhibit irradiation-induced regression of a 6-d Meth A tumor was abolished by treating the spleen cells with anti-Ly-1 antibody and complement, or anti-L3T4 antibody and complement. In contrast, treatment with anti-Ly-2 antibody and complement was without effect. These results indicate that γ irradiation causes regression by eliminating, or preventing the production of, $\text{Ly-1}^+, 2^-, \text{L3T4}^+$ suppressor T cells.

Irradiation-induced Tumor Regression Is Associated with Sustained Production of T Cell Effectors of Concomitant Immunity. It has been shown with the Meth A fibrosarcoma (4) and certain other tumors (3) that, in spite of their progressive growth, they nevertheless evoke in their hosts the generation of an underlying mechanism of concomitant immunity that is mediated by Ly-2^+ T cells. It has

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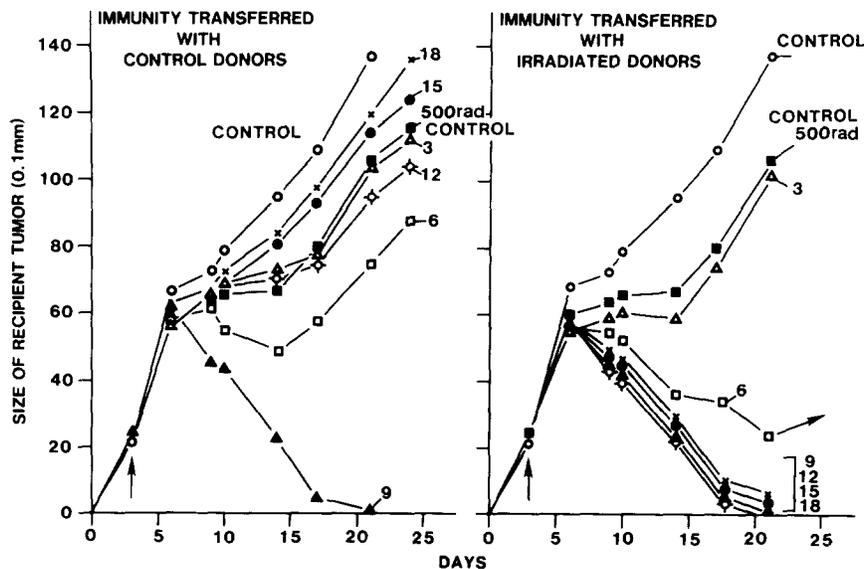


FIGURE 6. Irradiation-induced regression of the Meth A fibrosarcoma was associated with sustained production of lymphocytes that could passively transfer immunity. Shown is the growth of a test tumor in recipient mice that were infused (arrow) with one organ equivalent of spleen cells harvested from control (left), or irradiated donors (right) 3, 5, 9, 12, 15, or 18 d (numbers on graphs) after the donor tumor began growing. Radiation was given on day 5. It can be seen that it was possible to cause regression of the recipient tumor with one organ-equivalent of spleen cells from irradiated donors harvested at any time from day 6 until day 18 when the experiment was terminated. In contrast, tumor-bearing control donors rapidly lost protective lymphocytes after day 9. Thus, regression of the tumor after day 9 in irradiated mice (see Fig. 7) was associated with the sustained production of immune T cells. Means of five mice per group.

also been shown (4) that concomitant immunity is generated progressively between days 6 and 9 of tumor growth, but that it then undergoes progressive decay in concert with the progressive production of $Ly-1^+, 2^-$ suppressor T cells. On the basis of this knowledge it was possible to suggest that irradiation-induced tumor regression is caused by the ability of irradiation to preferentially eliminate suppressor T cells, thereby allowing the production of effector T cells to continue beyond day 9 of tumor growth. This would allow enough effector T cells to accumulate to cause tumor regression.

To determine whether γ irradiation causes a sustained production of effector T cells, tumor-bearing mice given 500 rad of γ radiation on day 5 of tumor growth were compared with control tumor bearers at progressive times in terms of the ability of one organ equivalent of their spleen cells to passively transfer immunity to recipients bearing a 3-d tumor. In this assay it was necessary to give the recipient mice 500 rad of γ radiation immediately before passive transfer, to enable donor spleen cells to express their antitumor function (4, 5).

It was found (Fig. 6) that progressive growth of the Meth A tumor in control mice resulted in the production of splenic T cells that could, on passive transfer, cause regression of a small Meth A tumor in irradiated recipients. Production of these T cells occurred between days 6 and 9 of tumor growth, after which they were rapidly lost. It was only on day 9 that the spleens of control mice contained

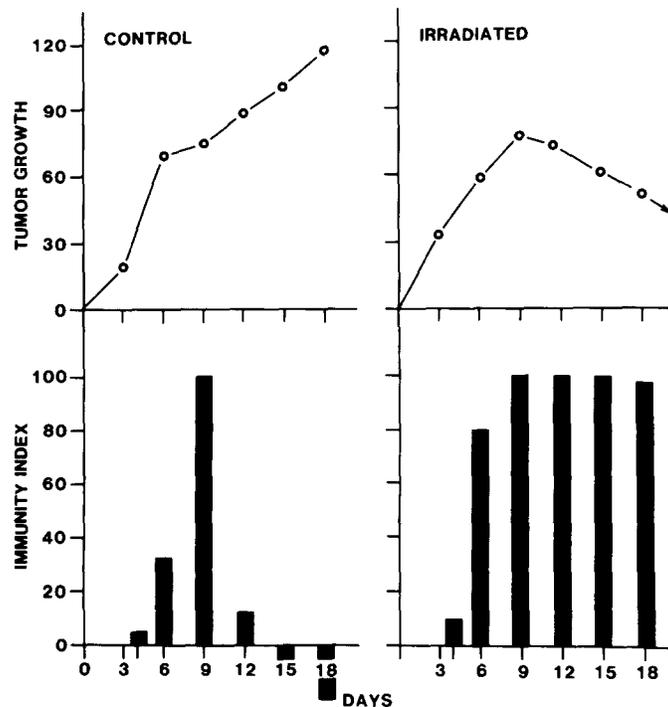


FIGURE 7. Results in Fig. 6 converted to changes against time in an index of immunity in tumor-bearing control donors (*left*) and tumor-bearing irradiated donors (*right*). The index of immunity was calculated by subtracting the size of the tumor growing in recipients that received donor spleen cells, from its size in control recipients on day 22 of the experiment. An index of 100 means that donor spleen cells caused complete tumor regression. This figure shows clearly that irradiated donors whose tumors underwent regression starting on day 9 retained lymphocytes that could cause regression of the recipient tumor until at least day 18.

enough sensitized T cells to cause complete regression of the recipient tumor. In contrast, donor mice that received irradiation on day 5 of tumor growth continued to generate effector T cells well beyond day 9. Indeed the spleens of irradiated tumor bearers contained effector T cells in numbers large enough to cause complete regression of the recipient test tumor until day 18, when the experiment was terminated. This was the case even though irradiation caused a severe reduction in the total number of spleen cells between days 5 and 18 of tumor growth.

Indeed, the cellularity of the spleens of mice irradiated on day 5 dropped from 1.5×10^8 to 1.7×10^7 on day 6, and increased progressively thereafter to reach 8×10^7 on day 18. In contrast, the cellularity of control spleens increased from 1.5×10^8 to 2.5×10^8 during the same time period. The sustained presence of effector T cells in irradiated mice was associated, after day 9, with progressive regression of the donor tumor. This is shown in Fig. 7, which also plots the above results in terms of changes in an index of concomitant immunity. Fig. 7 illustrates more clearly the consequences of irradiation on effector T cell production.

Irradiation-induced Tumor Regression Is Associated with Failure to Generate Sup-

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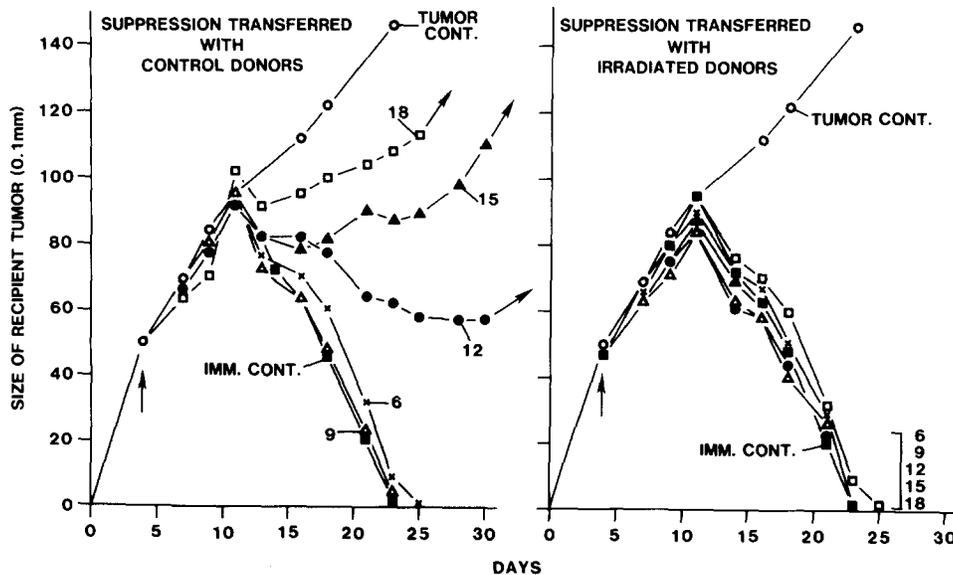


FIGURE 8. Irradiation-induced regression of the Meth A fibrosarcoma was associated with failure by the host to generate suppressor T cells. Shown is growth of a test tumor in TXB recipients that were infused (arrow) with the immune spleen cells alone, or immune spleen cells plus spleen cells from control (left), or irradiated tumor-bearing donors (right) harvested on day 6, 9, 12, 15, or 18 after implanting the donor tumor (numbers on individual graphs). Tumor regression caused by infusing immune cells alone (IMM CONT, ■) was suppressed by infusing spleen cells from control donors bearing a 12-, 15-, or 18-d tumor, with the degree of suppression increasing with size of the donor tumor. In contrast, spleen cells from irradiated tumor bearers (irradiated on day 5) failed to inhibit tumor regression, regardless of the time they were harvested. The tumor in the irradiated donors underwent regression starting on day 9 (see Fig. 9). Means of five mice per group.

pressor T Cells. It was shown in a preceding section that irradiation-induced regression of the Meth A fibrosarcoma can be inhibited by passive transfer of $\text{Ly-1}^+, 2^-, \text{L3T4}^+$ T cells from donors bearing an established Meth A tumor. In other words, regression can be inhibited by T cells with the same surface phenotype as T cells which, on passive transfer, can suppress the expression of adoptive immunity, and which are generated progressively after day 9 of tumor growth (4, 5). It was anticipated, therefore, that irradiation-induced regression of the Meth A tumor would be associated with a failure on the part of the host to generate these suppressor T cells. This was investigated by measuring, at progressive times of tumor growth, the capacity of one organ equivalent of spleen cells from control or irradiated mice to suppress, on passive transfer, the ability of one organ equivalent of passively transferred immune T cells to cause the regression of an established tumor in TXB test recipients, according to the standard suppressor assay (4, 5).

It can be seen in Fig. 8, in agreement with previous findings (4, 5), that control tumor bearers eventually generated splenic T cells that could, on passive transfer, suppress the ability of spleen cells from immunized mice to cause regression of a tumor in TXB test recipients. On a per spleen basis, these suppressor cells were generated progressively after day 9 of tumor growth. In contrast, the spleen of

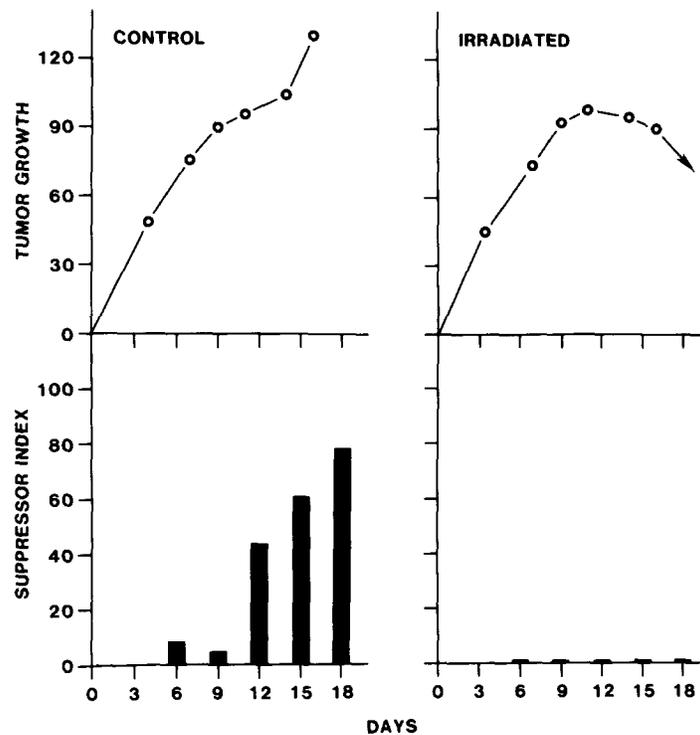


FIGURE 9. Results in Fig. 8 converted to changes against time in a suppressor index that was obtained by subtracting growth of the tumor in recipient of immune cells alone from its growth in recipients of immune cells plus suppressor cells on day 23 of the experiment. This figure clearly shows that, whereas normal tumor bearers acquired suppressor cells progressively from day 12 of tumor growth on, irradiated tumor bearers failed to acquire suppressor cells during the time course of the experiment.

tumor bearers irradiated on day 5 of tumor growth failed to acquire cells that could passively transfer suppression to adoptively immunized recipients. As shown in Fig. 9, the tumors in irradiated mice all began undergoing regression after days 9–12 of growth. Fig. 9 plots suppression in terms of changes against time in an index of suppression. It illustrates the difference in suppressor production between control and irradiated mice.

Early Irradiation Causes Enhancement of Tumor Growth. The foregoing results leave little doubt that irradiation-induced regression of the Meth A fibrosarcoma is immunologically mediated. They show that irradiation has no therapeutic effect against a tumor growing in a TXB host, and that regression can be prevented by infusion, after irradiation, of T cells from a tumor-bearing donor. Additional evidence that the dose of radiation used had no direct effect on growth of the tumor is shown by the results of experiments that determined the consequences of giving irradiation at different stages of tumor growth. Figs. 10 and 11 illustrate, with the Meth A fibrosarcoma and SA1 sarcoma, respectively, that whole-body exposure to 500 rad of radiation shortly after implanting tumor cells resulted in enhancement of growth of the tumors that emerged. In contrast, exposure to the same dose of irradiation after the tumors had become palpable

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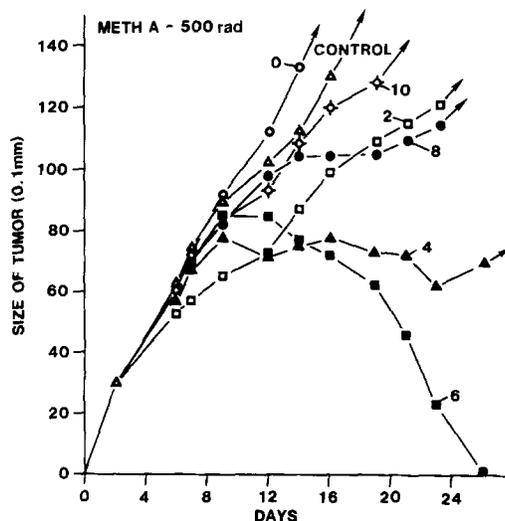


FIGURE 10. Therapeutic effect of 500 rad of γ radiation given at different days (numbers on individual graphs) of growth of the Meth A fibrosarcoma. In this experiment, radiation was most therapeutic when given on day 6, with therapeutic effectiveness falling off on either side of day 6. Note, however, that when radiation was given several hours after implanting tumor cells (time 0) it caused enhancement of tumor growth. Means of five mice per group.

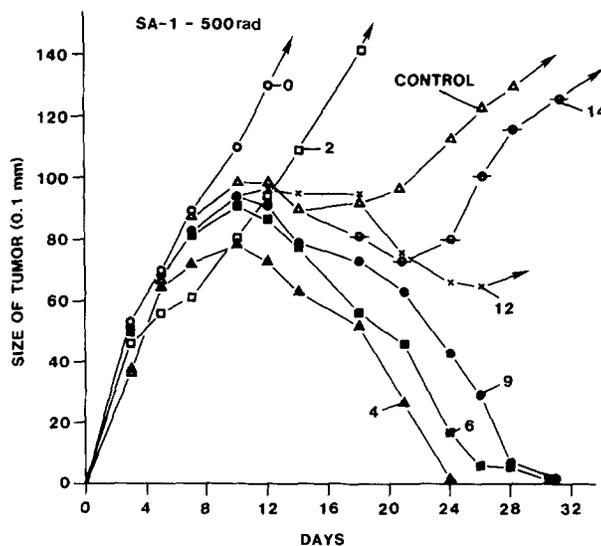


FIGURE 11. Same as Fig. 9 except with SA1 sarcoma. With this tumor, irradiation caused complete regression when given on days 4, 6, or 9 of tumor growth (numbers on individual graphs). This figure better illustrates that irradiating too early (during the first 2 d) after tumor implantation caused enhancement of tumor growth. Means of five mice per group.

resulted in complete tumor regression. Thus, the ability of a host to respond immunologically to its immunogenic tumor changes from being highly radiosensitive to being highly radiostimulative during the first 4 d or so of tumor growth. It can be seen, moreover, that the therapeutic effect of irradiation decreased

progressively after day 6 of tumor growth, and that the SA1 sarcoma was more responsive overall to irradiation than the Meth A.

Discussion

The results reported here serve to confirm results published by other authors (1) showing that exposure of mice bearing a palpable immunogenic tumor to a sublethal dose of ionizing radiation can cause the tumor to undergo partial or complete regression, depending on its immunogenicity. However, in disagreement with a key finding by the same authors, this study failed to find evidence that T cells from normal donor mice can inhibit the therapeutic effect of irradiation. On the contrary, to inhibit irradiation-induced regression of the Meth A fibrosarcoma, it was necessary to infuse the irradiated host with T cells from donors bearing a progressive Meth A tumor. Be this as it may, the results reported here and by others (1) support the interpretation that the therapeutic effect of irradiation is in some way dependent on the elimination of radiosensitive suppressor T cells, or their precursors. Any suggestion that irradiation-induced tumor regression is the result of the direct destructive action of radiation on tumor cells can be discontinued on the basis of the additional finding that irradiation failed to cause the regression of tumors growing in TXB mice that could not generate an antitumor immune response. Therefore, irradiation-induced regression of an established tumor is a convincing example of successful immunotherapy.

A major contributor of the present study to an understanding of this immunotherapy is that it provides direct evidence that irradiation-induced regression is associated with failure of the host to generate suppressor T cells on the one hand, and with a sustained production of effector T cells on the other. In this connection, preceding studies from this laboratory have shown (discussed in references 2 and 3) that, because progressive growth of immunogenic tumors can evoke the generation of an underlying state of concomitant immunity, the therapeutic action of any given agent against an established tumor needs to be interpreted in terms of the influence of the agent on this underlying immune response. It is known that the response results in the progressive generation, between days 6 and 9 of tumor growth, of Ly-2⁺ T cells that, on passive transfer, can cause regression of a small 3-d tumor in irradiated recipients (4, 5). After about day 9, these protective T cells are progressively lost and this is temporally associated with the progressive acquisition of Ly-1⁺, 2⁻ suppressor T cells. The suppressor function of these suppressor T cells is measured in terms of their capacity to suppress, on passive transfer, the ability of passively transferred T cells from immunized donors to cause the regression of an established tumor in TXB test recipients (4, 5). It was hypothesized, on the basis of these and other published results (4, 5), that immunogenic tumors grow progressively because the immune response they evoke in their immunocompetent hosts is down-regulated by suppressor T cells before enough effector T cells are generated to cause tumor regression. The results presented here are entirely consistent with this hypothesis. They provide convincing evidence that if suppressor T cells are removed, a large enough number of effector T cells can be generated to cause tumor regression. Thus, failure to generate suppressor T cells after 500 rad of

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whole-body irradiation results in a sustained production of effector T cells that can, on passive transfer, cause regression of a test tumor in appropriate recipients. It is interesting in this regard that exposure to radiation on day 6 of tumor growth does not result in the onset of tumor regression until after about day 9, when concomitant immunity to the Meth A fibrosarcoma normally begins to undergo progressive decay. This indicates that tumor regression depends on the uninterrupted production of effector T cells beyond day 9, rather than on an accelerated production of effector T cells immediately after irradiation. In other words, a given amount of time is required for therapeutic numbers of effector T cells to accumulate, even in the absence of suppressor T cells.

In the case of those tumors that fail to undergo regression in response to whole-body irradiation, it is apparent that they are not immunogenic enough to cause the generation of a therapeutic number of effector T cells, even in the absence of suppression. If this proves to be true, it would mean that the negative regulatory influence of tumor-induced suppressor T cells need not explain the escape of all immunogenic tumors from immunity. This possibility is currently under study in this laboratory with the P815 mastocytoma and P388 lymphoma.

Regardless of the reason why some tumors fail to undergo regression in response to irradiation, there remains the central problem of explaining, in the case of those that do, why irradiation eliminates suppressor T cells without effecting the production and function of effector T cells. Obviously effector T cells are not destroyed, otherwise irradiation would have no therapeutic effect on the tumor. The problem, then, is not to explain the sensitivity of suppressor T cells or their precursors to the dose of radiation used, because it is well documented (reviewed in 8) that suppressor T cells in general are more radiosensitive than other lymphocytes, and it is known (6) that the suppressor T cells generated in response to growth of the Meth A fibrosarcoma and other tumors under study in this laboratory are destroyed *in vivo* by 500 rad of γ radiation. Rather, the problem is to explain why effector T cell production is not abolished by the same dose of radiation. To this end, it is possible to draw on published evidence (9) showing that, in contrast to resting lymphocytes that are highly radiosensitive, B lymphocytes and T lymphocytes that are activated by mitogens, or T lymphocytes that are activated by alloantigens, are relatively radioresistant, in that they survive and function for some time, even after exposure to 1,000 rad of x radiation. Indeed, it has been shown (9) that continuous lines of cytolytic and Th cells can proliferate to a surprising extent after exposure to high doses of x rays. This evidence is relevant to the demonstration here (Figs. 10 and 11) that, whereas irradiation during the first day or so after implanting tumor cells is highly immunosuppressive, in that it causes enhanced growth of the tumor that emerges, irradiation 3–5 d later is highly immunoenhancing in that it causes immunologically-mediated tumor regression. It is suggested that this conversion from being radiosensitive to radioaugmentable signifies the onset of induction of the antitumor immune response and the consequential activation of specifically sensitized T cells to a radioresistant cycling state. Unlike most other mammalian cells that are radioresistant in a resting state, and radiosensitive while cycling, it is apparent that lymphocytes are radiosensitive when resting and radioresistant while cycling. Obviously, because this does not apply to tumor-

induced suppressor lymphocytes, there must be physiological differences between activated effector T cells and activated suppressor T cells. There is a need to discover more acceptable immunotherapeutic modalities to take advantage of these physiological differences.

Summary

The results of this study confirm results published by others (1) by showing that sublethal whole-body irradiation of mice bearing immunogenic tumors can result in complete tumor regression. The results show, in addition, that irradiation-induced tumor regression can be prevented by infusion, after irradiation, of Ly-1⁺,2⁻,L3T4⁺ suppressor T cells from the spleens of donors bearing an established tumor, but not by infusion of normal spleen cells. This evidence, plus the demonstration that irradiation fails to cause regression of tumors growing in immunocompetent mice, is consistent with the hypothesis that irradiation-induced regression is immunologically mediated, and that it depends on the ability of irradiation to preferentially eliminate suppressor T cells. By using passive transfer assays to measure the production of effector T cells and suppressor T cells against time of tumor growth, it was shown that irradiation of tumor-bearing mice on day 5 of tumor growth resulted in a failure to generate suppressor T cells on the one hand, and in a sustained production, effector T cells on the other. In other words, irradiation prevented the concomitant antitumor immune response from being downregulated by suppressor T cells. However, giving radiation on day 1 of tumor growth, in contrast to giving it 3–6 d later, caused immunodepression and enhancement of tumor growth. This is in keeping with published evidence showing (9) that, whereas resting effector T cells are highly radiosensitive, antigen-activated effector T cells are relatively radioresistant. It is suggested that the radioresistance of activated effector T cells, coupled with the radiosensitivity of activated suppressor T cells, is the reason for the selectivity of ionizing radiation for suppressor T cells and why a tumor needs to be palpable to undergo regression in response to radiation therapy.

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