Interleukin 1 Protects against the Lethal Effects of Irradiation of Mice but has No Effect on Tumors in the Same Animals (42884)

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Abstract. **Interleukin 1 (IL-1) is a radioprotector of bone marrow and is cytotoxic to some tumor cells. This investigation examines these two properties in the same host animals and gives evidence of radioprotection against localized x-irradiation of the head and neck region. By LD_{50} analyses, recombinant human IL-1 (100 ng/mouse, approximately 3 μg/kg) was found to be radioprotective against whole-body irradiation for both C3H/Km and C57BL/Ka mice. The combined potency ratio for the two strains was 1.07 (95% confidence limit: 1.02–1.12). It was also radioprotective against the injury leading to acute lethality resulting from localized head and neck irradiation of C3H/Km mice; 100 ng of IL-1/mouse produced a potency ratio of 1.05 (95 confidence limit: 1.03–1.07). However, two tumors that originated in C3H/Km mice, RIF-1 and SCCVII, showed neither *in vitro* nor *in vivo* response to IL-1. Also, there was no IL-1-induced reduction in *in vivo* growth of the RL12NP lymphoma in C57BL/Ka mice.** [P.S.E.B.M. 1989, Vol 191]

Although interleukin 1 (IL-1) has for the most part been studied in the context of inflammation, evidence is accumulating that it can also contribute to the regulation of hemopoiesis. Neta *et al.* (1) found that recombinant IL-1 can protect mice against radiation-induced bone marrow death. The mechanism by which this occurs is not fully understood. IL-1 induces the production of granulocyte-macrophage and granulocyte colony-stimulating factors and burst-promoting activity in cultures of bone marrow cells and cloned bone marrow stromal cells (2–4) and *in vivo* (5). IL-1 appears to be the same as hemopoietin 1, which has synergistic effects with granulocyte-macrophage colony-stimulating factor and macrophage colony-stimulating factor, increasing the proliferation of early granulocyte-monocyte precursors (6). These findings suggest that IL-1 may be useful in the clinic to minimize toxicity from bone marrow injury that may be encountered in cancer radiotherapy.

Although hemopoietic toxicity is a major problem only after whole-body irradiation which is used infrequently in the clinic, the potential for reducing such toxicity with IL-1 raises the question of whether this lymphokine has comparable effects on other normal tissues. For this reason, we have investigated whether IL-1 radioprotection can be achieved when radiation is localized to the head and neck region. It is essential for the usefulness of IL-1 as an adjunct to radiotherapy that there be no increase in proliferation of tumor cells nor tumor cell protection from ionizing radiation damage. Most available evidence suggests that IL-1 limits, rather than augments, tumor growth (7–12). Nevertheless, it is important to examine tumor response under the same conditions in which IL-1 protects the bone marrow or other normal cells. We have, therefore, assessed the extent of radioprotection by IL-1 in two strains of mice, C3H/Km and C57BL/Ka, and examined its effects on the growth and sensitivity to x-irradiation of two tumors of the strain C3H/Km and on the growth of the RL12NP lymphoma of the C57BL/Ka strain.

Materials and Methods

IL-1. Recombinant human IL-1α and IL-1β (Immunex Co.), in saline/sodium citrate solution at pH 7.0, were diluted appropriately either with Waymouth's medium supplemented with 15% fetal calf serum for use in tissue culture or with bacteriostatic nonpyrogenic normal saline (Abbott Laboratories) for use in mice *in vivo*.

Animals. Male C3H/Km and C57BL/Ka mice,

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from 2½ to 3 months of age unless otherwise specified, were used in this study and were produced in our Stanford departmental mouse colony, which is maintained routinely under pathogen-free defined flora conditions. All cages were equipped with filter tops and were supplied with autoclaved pasteurized mouse chow and sterile water. For IL-1 dose-response experiments, LD_{50}, and RL12NP lymphoma studies, the mice were randomized after treatment and housed two or three per cage. Deaths were recorded twice daily during the week and once per day on weekends.

Tumors. Tumor cell lines in C3H/Km mice were either RIF-1 (13), a radiation-induced murine fibrosarcoma, or SCCVII (14), a murine squamous cell carcinoma. The tumor cell line, designated RL12NP, was derived from a radiation-induced lymphoma (15) in a C57BL/Ka mouse.

Irradiation. All irradiations were performed with a 250-kVp x-ray unit (HVL, 1.0 mm Cu). Cells in 60-mm plastic tissue culture petri dishes were irradiated (at room temperature) in a nylon carrier 50 cm from the source and at a dose rate of approximately 1.5 Gy/min. For tumor regrowth delay assays, unanesthetized mice were placed in lead boxes with only their right hind limb protruding into the field of radiation. The distance from the source to the skin was 33 cm and the dose rate was approximately 2.5 Gy/min. For whole-body irradiation, unanesthetized mice were enclosed within individual stalls radiating from the center of an octagonal shaped Plexiglas box at a source to skin distance of 50 cm and irradiated at a dose rate of approximately 1.5 Gy/min. For head and neck irradiation, unanesthetized mice were restrained within Plexiglas tubes affixed to 3-mm lead shields with portals for radiation exposure (everything craniad of the manubrium) at a source to skin distance of 36 cm and at a dose rate of approximately 2.4 Gy/min.

Radiation LD_{50}. All LD_{50} values in this article are based on deaths that occurred within 30 days after the completion of treatment, whether the treatment was partial- or whole-body irradiation. C3H/Km and C57BL/Ka mice were allocated to five groups of 16, and at approximately 20 hr before whole-body irradiation, one-half of the mice were injected intraperitoneally with 100 ng of IL-1α or IL-1β. The two strains were tested in separate experiments. Based on an average mouse weight of approximately 35 g, the dose of IL-1 was approximately 3 μg/kg. For head and neck irradiation studies, there were nine C3H/Km mice per group, and the IL-1β dose given 20 hr before irradiation was 100 ng. Final potency ratios were determined by multiple logistic regression analysis.

IL-1 Dose Response. In order to examine the influence of the IL-1 dose on radioprotection, C57BL/Ka mice, 6–7 months of age, in groups of eight were injected intraperitoneally with 0–7 μg/kg Il-1β in saline carrier (as described above) 20 hr before whole-body irradiation with a single dose of 7.8 Gy. The degree of radioprotection was assessed from the reduction in the number dead by 30 days later.

***In Vitro* Tumor Cell Survival.** RIF-1 cells or SCCVII cells were plated in Waymouth's (GIBCO) medium supplemented with 15% fetal calf serum at a concentration of 1×10^5 cells per 60-mm plastic tissue culture dish (Lux). Cells were incubated in a humid atmosphere at 37°C in 5% CO_2 in air. On the third day after plating, 2 ml of the medium were replaced with medium containing IL-1α sufficient to produce final concentrations of 0, 2, or 4 ng/ml per dish. Approximately 20 hr after beginning the IL-1α treatment, the culture dishes were x-irradiated with either 0, 3, 6, 9, 12, or 15 Gy and then returned to the incubator. At 2 hr after the completion of each irradiation, the medium in each dish was removed by aspiration, and 4 ml of 0.05% trypsin in Hanks' solution was added as a wash and then removed promptly. The cells were then incubated at 37°C for 15 min, with an additional 1 ml of trypsin. For macrocolony formation, single-cell suspensions in Waymouth's medium were prepared and appropriate dilutions were plated in either 60-, 100-, or 150-mm plastic tissue culture dishes. For each x-ray dose, cells were plated at three dilutions with four replicate plates per dilution. Two weeks after plating, the colonies were stained with crystal violet and those colonies containing more than 50 cells were counted to determine the surviving fraction.

***In Vivo* Tumor Response.** RIF-1 or SCCVII tumors were implanted intramuscularly by inoculating 2×10^5 cells in the gastrocnemius muscles of the right hind limbs of C3H/Km mice. The inoculated and contralateral legs were measured in two orthogonal cross-sectional dimensions, and each tumor area was computed by subtracting the product of the measurements of the control leg from that of the tumor-bearing leg. Experiments were begun 2 weeks after inoculation, when the mean tumor area was 45 mm^2. Groups of 10 mice were irradiated with single doses of 15, 20, 25, or 30 Gy. Five mice from each dose group were injected ip with 100 ng of IL-1α approximately 20 hr before irradiation. Tumor areas were determined thrice weekly until each tumor had exceeded three times its original area. Using curvilinear regression analysis, a regrowth curve for each tumor was determined in order to calculate the time to achieve three times the area at the time of treatment. Values for unirradiated control tumors were subtracted to determine the increase in growth delay as a function of radiation dose.

The intraperitoneal inoculation of 5×10^5 to 1×10^7 RL12NP cells in C57BL/Ka mice was preceded at 20 hr by intraperitoneal injection of 0 or 100 ng of IL-1α. Each treatment group contained six mice. Deaths were recorded as described above and the length of time after treatment was related to the number of cells injected.

Results

Radioprotection. The data for LD_{50} analyses in C57BL/Ka and C3H/Km mice treated with and without IL-1α before whole-body irradiation are shown in Figure 1. The LD_{50} values, as indicated for the two strains, were not different, and analysis by multiple logistic regression for the combined data gives a potency ratio of 1.07 (95% confidence limit: 1.02–1.12) for IL-1α. (The *potency ratio* is *LD_{50} with drug added/LD_{50} for irradiation only.*) In another experiment using IL-1β in C57BL/Ka mice, a similar potency ratio, 1.13 (95% confidence limit: 1.05–1.21), was obtained.

Table I lists the proportion of C57BL/Ka mice that were dead within 30 days after 7.8 Gy x-irradiation preceded 20 hr earlier by IL-1β injection. There may have been a small amount of radioprotection for doses as low as 0.01 to 0.1 μg/kg, although significant differ-

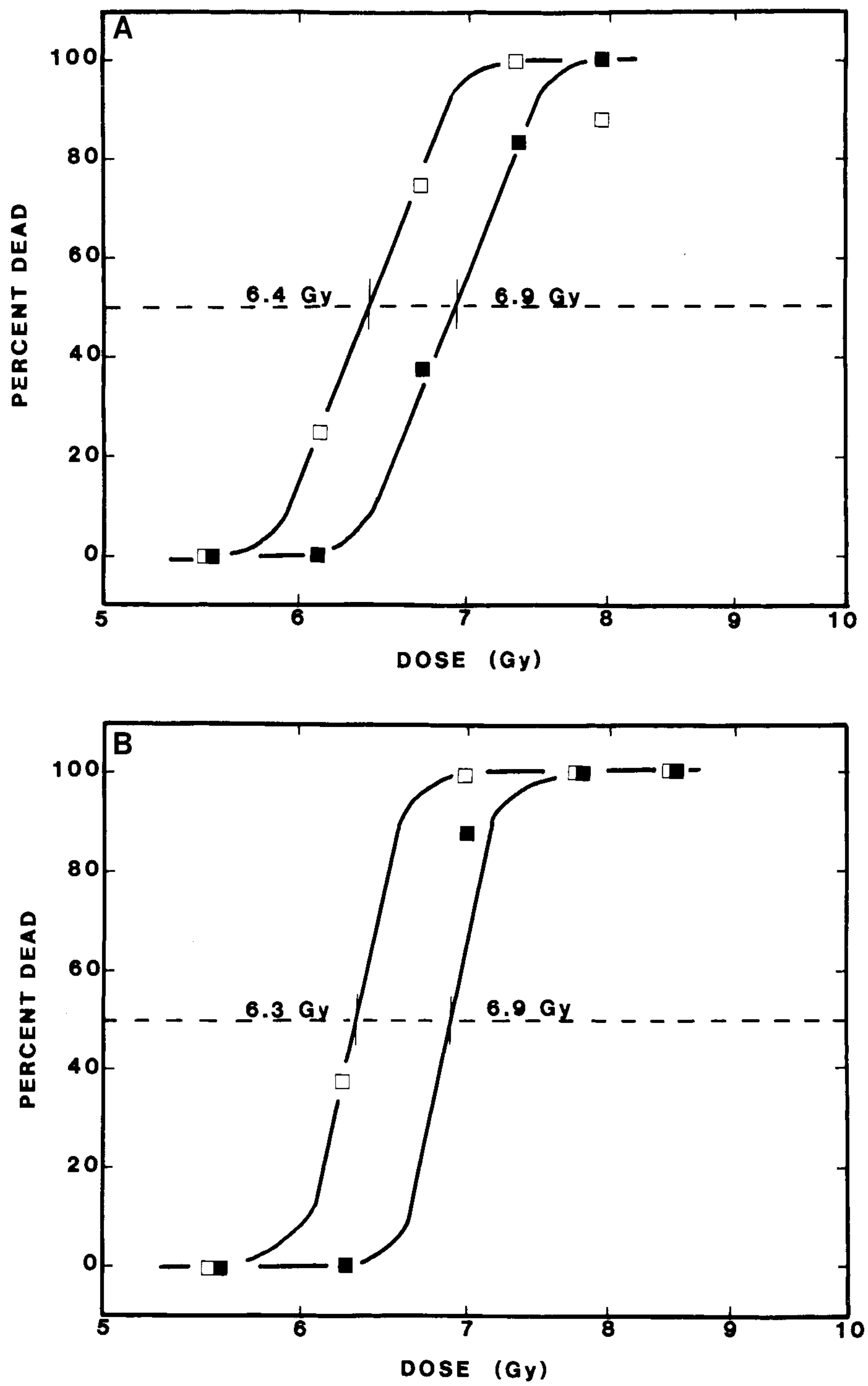

Figure 1. Protection against whole-body x-ray lethality in C57BL/Ka (A) and C3H/Km mice (B). Open symbols denote irradiation alone and closed symbols represent 100 ng of IL-1α given 20 hr before irradiation. The LD_{50} values are shown in the body of the graph.

ences ($P \leq 0.05$) in survival above control levels were observed only for doses of at least 0.25 μg/kg. The survival in this experiment was different from that shown in Figure 1A, but this presumably can be attributed to the marked difference in animal age (6–9 vs 2½–3 months).

Figure 2 shows the mortality data for C3H/Km mice treated with and without 100 ng of IL-1β 20 hr before localized head and neck irradiation. The LD_{50} values, as indicated, gave a potency ratio of 1.05 (95% confidence limit: 1.03–1.07) by multiple logistic regression. From the changes in body weight, i.e., a decrease of approximately 20% by Day 7 and 40% at Day 11, it seems apparent that the mice died of an "oral death syndrome" (16).

Table I. Proportion of Recombinant IL-1 Pretreated C57BL/Ka Mice Dead at 30 Days after 7.8 Gy Whole-Body Irradiation

Dose of IL-1 (μg/kg)	Deaths[a]
0.00	14/16 (87.5)
0.01	5/8 (62.5)
0.05	5/8 (62.5)
0.10	5/8 (62.5)
0.25	2/8 (25.0)
0.50	2/8 (25.0)
0.75	1/8 (12.5)
1.00	6/16 (37.5)
2.00	3/8 (37.5)
3.00	4/8 (50.0)
4.00	3/8 (37.5)
5.00	2/8 (25.0)
6.00	2/8 (25.0)
7.00	2/8 (25.0)

[a] No. dead/no. treated (%).

Tumor Response to IL-1. Treatment with IL-1α *in vitro* had little or no effect on the plating efficiency of RIF-1 (0.7845 ± 0.0486) or SCCVII (0.8418 ± 0.0249) tumor cells. From the radiation survival curves of RIF-1 and SCCVII tumor cells (Fig. 3), it can be seen that treatments with 2 and 4 ng/ml IL-1α had no effect on the survival to irradiation of clonogenic tumor cells.

As shown in Figure 4, RIF-1 and SCCVII tumors allowed to remain *in situ* after irradiation manifest growth delay which increases with radiation dose. Treatment with IL-1α (100 ng/mouse) did not change the growth delay of either tumor. In each case, the slopes were not significantly different, nor was the slight separation of the two best fitting regression lines for the RIF-1 tumor.

Figure 5 indicates that mice given IL-1α before inoculation with RL12NP lymphoma cells tended to die earlier than those receiving cells alone, but the difference of approximately 2 days was not significant ($P > 0.05$).

Discussion

In this study we have examined both the radioprotection and tumor cytotoxicity that have been attributed to the action of IL-1. Both IL-1 species, α and β,

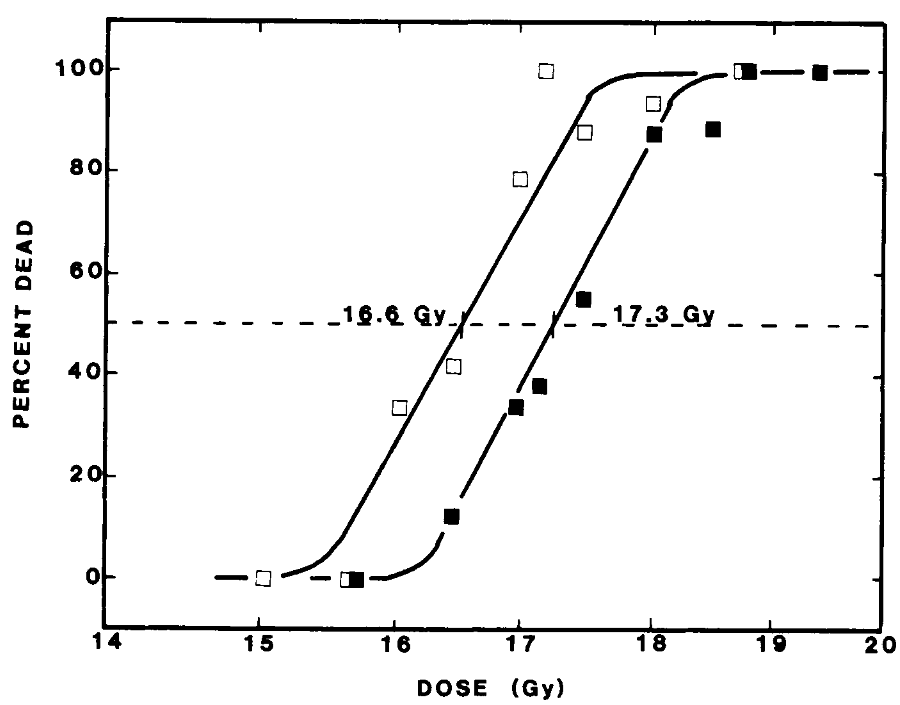

Figure 2. Radioprotection against head and neck x-ray lethality in C3H/Km mice. Open symbols denote irradiation alone and closed symbols 100 ng of IL-1β given 20 hr before irradiation. The LD_{50} values are shown in the body of the graph.

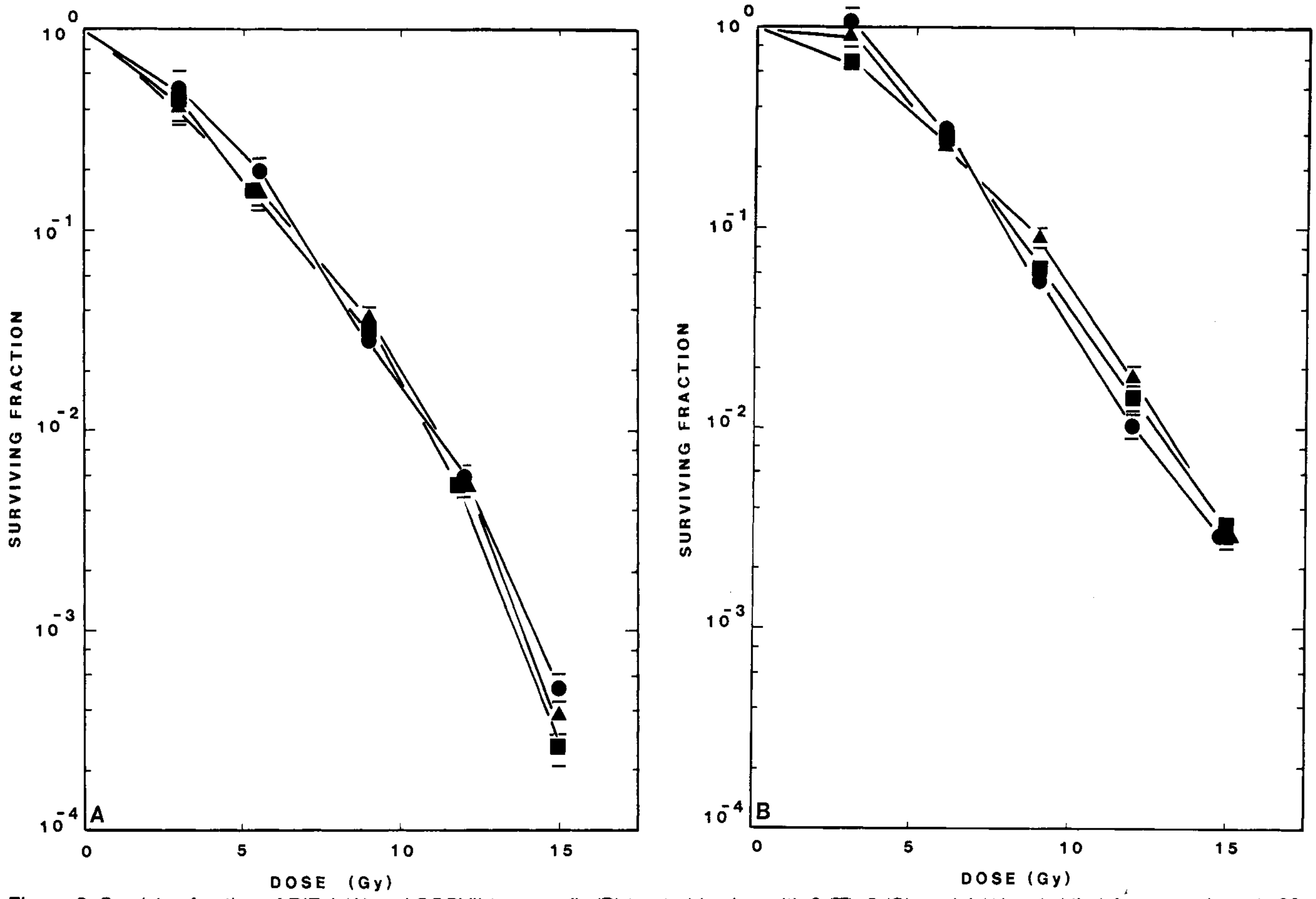

Figure 3. Surviving fraction of RIF-1 (A) and SCCVII tumor cells (B) treated *in vitro* with 0 (■), 2 (●), and 4 (▲) ng/ml IL-1 for approximately 20 hr before x-irradiation. Error bars for standard deviations shown only for errors larger than symbols.

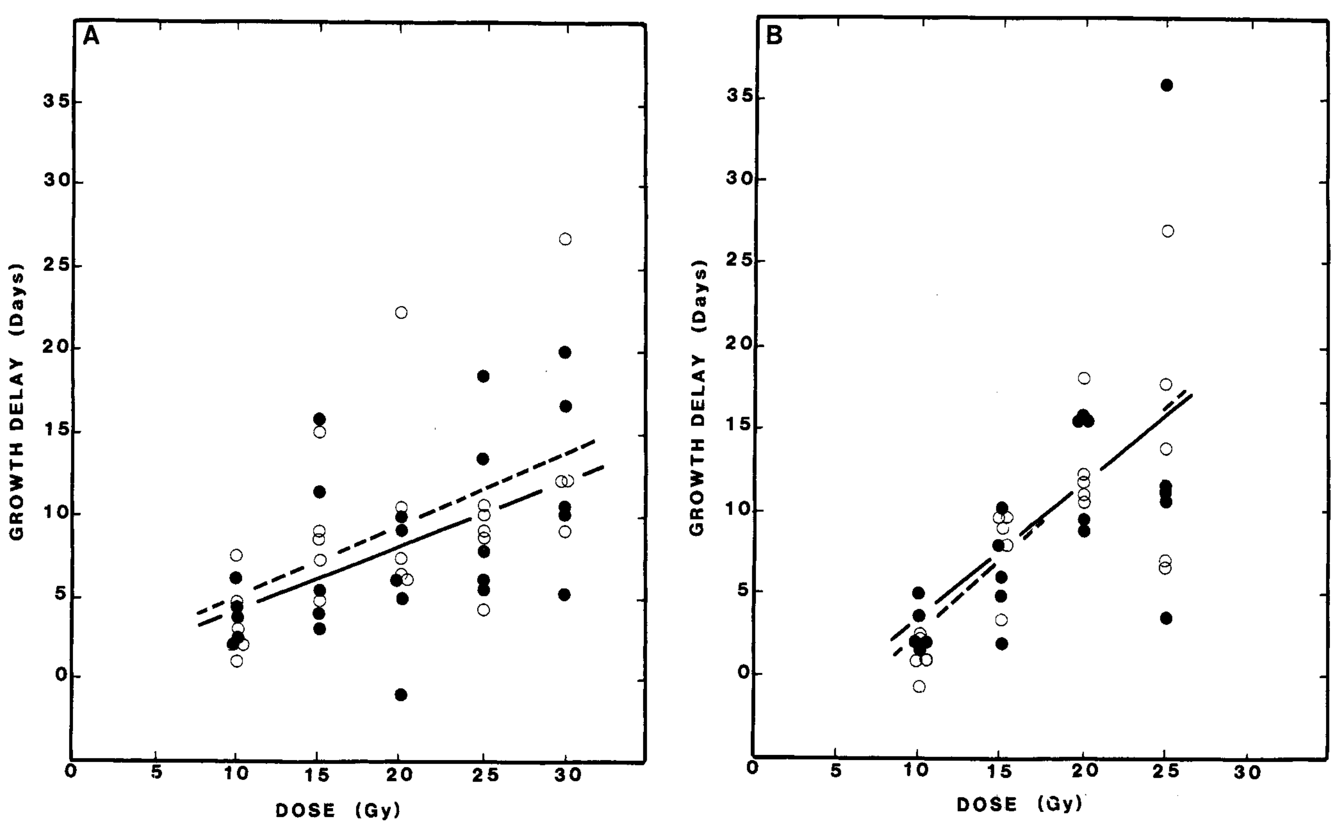

Figure 4. Growth delay of RIF-1 (A) and SCCVII tumors (B) treated *in vivo* with (●) and without (O) 100 ng ip IL-1 injection at approximately 20 hr before irradiation. Solid line is with and dashed line is without IL-1. Each symbol represents an individual mouse.

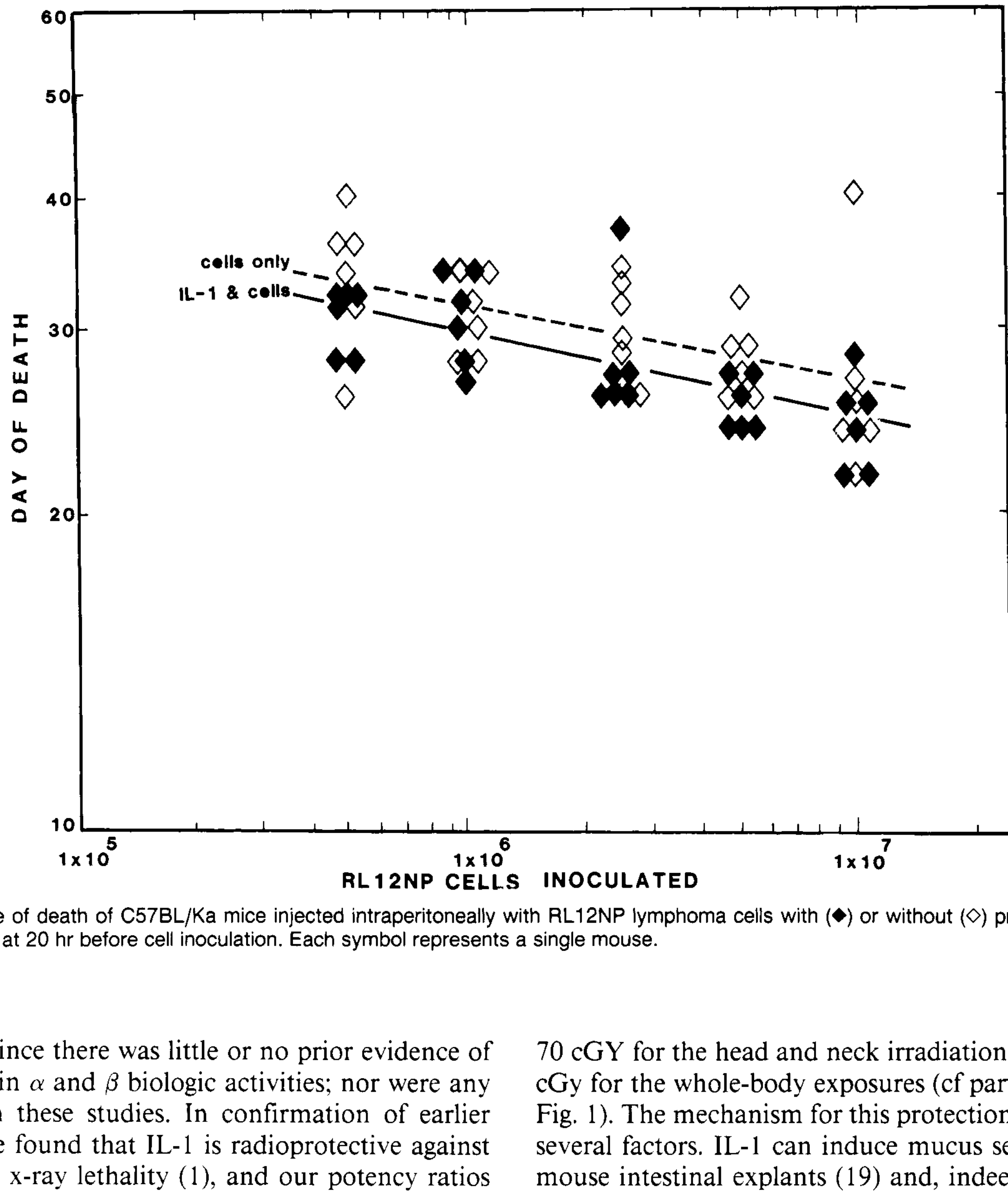

Figure 5. Time of death of C57BL/Ka mice injected intraperitoneally with RL12NP lymphoma cells with (♦) or without (◇) pretreatment with 100 ng of IL-1 at 20 hr before cell inoculation. Each symbol represents a single mouse.

were used since there was little or no prior evidence of differences in α and β biologic activities; nor were any observed in these studies. In confirmation of earlier findings, we found that IL-1 is radioprotective against whole-body x-ray lethality (1), and our potency ratios approximate those in published studies in which complete LD_{50} curves (17) were determined. It is of note, though, that our murine substrains, C3H/Km and C57BL/Ka, were protected equally, whereas the protection afforded mice of the C3H/HeN strain was only a fraction of that for C57BL/6 mice (17). Although these differences could be taken as evidence for variation in the response to IL-1, one cannot overlook the importance of the possible presence of pathogens and/or different flora among different animal colonies and even between strains in the same institution. It is well known that irradiation can enhance susceptibility to infection, and this can account for different sensitivities of different strains (18).

Of further importance is our evidence that IL-1 is protective against the lethal injury incurred by localized head and neck irradiation. Although the potency ratio of 1.05 obtained here is less than that seen in the whole-body lethality experiments, the dose elevation is greater: 70 cGY for the head and neck irradiation vs 50 and 60 cGy for the whole-body exposures (cf parts A and B of Fig. 1). The mechanism for this protection may involve several factors. IL-1 can induce mucus secretion from mouse intestinal explants (19) and, indeed, one of the causes of death among the head and neck irradiated mice is diffuse mucosal damage in the oral cavity (20). There may be other factors involved that are related to the kind of radioprotection afforded by IL-1 against lethal bone marrow damage. Recently, recombinant IL-1 has been shown to cause bone marrow cell enlargement and an increase in cell cycling capacity (21) and to produce an accelerated recovery of hemopoiesis following whole-body irradiation (22). In view of the role of IL-1 in the production of various colony-stimulating factors (23–25), it is likely that these phenomena involve the induced formation of other cytokines that then act synergistically with their inducer.

IL-1 may exert a toxic action directly (9, 11) or indirectly by its mediation in the stimulation of antitumor immunity (7, 8, 10). It is therefore relevant that neither the RIF-1 (13) nor the SCCVII (Kallman, unpublished data) tumor have been shown to be immunogenic as tested by standard methods. If IL-1 had influenced directly the growth of tumor cells or their sensitivity to ionizing radiation, this would have been manifested in our experiments. However, it should be noted that tumor response to IL-1 can vary with tumor cell line, thus indicating some degree of specificity for target cell lineage (10). Although most previous reports of IL-1 antitumor activity have been based on *in vitro* systems, at least one study has shown effects *in vivo* (12), and we are currently investigating the potential effect of IL-1 on the radiation sensitivity of the EMTC tumor which is known to have become immunogenic in mice of the BALB/c strain from which it arose.

Our studies showing that IL-1 can act as a radioprotective agent for critical radiation dose-limiting normal tissues under conditions when it is without demonstrable effects on tumor cells are most encouraging. This cytokine may be of real practical value in certain kinds of tumor therapy. It would be of obvious utility when the whole body is exposed and the dose of radiation that can be administered is limited by bone marrow toxicity. Insofar as whole-body irradiation has become increasingly popular in the treatment of disseminated lymphomatous disease, inclusion of IL-1 in the treatment protocols may even permit increasing the x-ray dose, thereby achieving a higher tumor cell kill in the presence of enhanced normal hemopoietic cell survival. It is intriguing and perhaps more significant that its dose reduction property is not confined to the stem cells of the blood-forming system, and this suggests its potential usefulness when large fields involving, for example, the head and neck, must be treated. It is, of course, necessary to ascertain whether IL-1 can provide similar protection when radiation is delivered in clinically relevant multiple fractions and that study is currently underway.

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