# Postponement of Murine Radiogenic Leukemia by Manipulation of the Preleukemic State* (33774)

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The development of leukemia in the irradiated mouse may be prevented or postponed by removing hematopoietic organs before overt disease is present (1, 2). Since hematopoietic cells can be eliminated systemically with antimetabolites, we attempted to postpone radiogenic leukemias by treating irradiated mice chemically or by exposing them to additional radiation.

*Methods.* One hundred and seventy-three male and female RFM/U mice (60–90 days old) were whole-body irradiated and randomly assigned to one of three groups during a 4-month period. Radiation factors were 350 rads of 250-kV X-rays (half value layer 1.5 mm Cu) and dose rate in soft tissue, approximately 48 rads per min. The first of the three groups received no further treatment. The second group, including 58 mice, received the same radiation dose again 70 days after the first irradiation. A third group of 58 mice received 6-mercapto-purine (25 mg/kg/day/mouse) 70 days after irradiation for 10 consecutive days.

Survival probability was estimated by the product-ratio method of Kaplan and Meier (3). Nonleukemic deaths were considered losses, on the assumption that the risks of competing causes of death are additive. In our study, we departed somewhat from this assumption, partly to determine the robustness of the product–ratio method. The order of the three groups relative to survival probability was unchanged by this testing of robustness. To test the significance of a change in expected life-span without making any assumption about the additivity of competing causes of death, we considered only mice which had been in the study for more than 425 days. We then used $t$ tests to compare the average ages of mice in the three groups at death (without distinguishing leukemic from nonleukemic causes). For our purposes, mice surviving more than 425 days were considered to have died at 425 days.

*Results.* Beginning approximately 90 days after the first irradiation, mice of all three groups developed leukemias, including thymic lymphoma, myeloid leukemia, extrathymic lymphoma, and undifferentiated leukemias. The total incidence of all leukemias is shown in Table I. Fig. 1, showing the derived probability functions, suggests that the animals in groups 2 and 3 treated during the preleukemic interval developed leukemia later than those in group 1, which were not treated during this interval. Only at the end of the life-span was the probability of dying from leukemia the same in all three groups. Hence, when nonleukemic causes of death were eliminated, the expected life-span was longer in mice treated during the preleukemic interval. The survival time of mice reirradiated 70 days after the first irradiation (group 2) was significantly longer than that of either group 1 or 3 ($p > 0.02$, 2-tailed). We concluded that the later development of leukemia in this group was real. However, the survival time of mice given 6-mercaptopurine was not distinguishable from the survival time in group 1. The later onset of leukemogenesis apparent in this group may not be significant, or it may be balanced by the increase in death rates from other causes.

*Discussion.* The interest of these observations is twofold. They reveal basic physiopathologic aspects of the preleukemic state and they may provide a basis for the prophylaxis of leukemia.

Haran-Ghera and Peled (4) postulated that immune suppression associated with acute radiation injury results in increased

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TABLE I. Incidence of Leukemia and Nonleukemic Deaths in the Three Groups of Mice.

| Days after initial irradiation | No additional treatment after initial irradiation | | Treatment after initial irradiation | | | |
|---|---|---|---|---|---|---|
| | | | 6-Mercapto-purine | | Irradiation | |
| | No. present | No. dying from leukemia | No. present | No. dying from leukemia | No. present | No. dying from leukemia |
| 0–25 | 57 | | 58 | | 58 | |
| 25–50 | 57 | | 58 | | 58 | |
| 50–75 | 57 | | 58 | | 58 | |
| 75–100 | 57 | 1 | 58 | | 58 | |
| 100–125 | 56 | | 52 | 1 | 58 | 1 |
| 125–150 | 56 | 2 | 49 | 1 | 54 | |
| 150–175 | 54 | 5 | 47 | 1 | 45 | |
| 175–200 | 47 | 6 | 44 | | 44 | 2 |
| 200–225 | 39 | 4 | 42 | 5 | 42 | 1 |
| 225–250 | 35 | 5 | 32 | 3 | 39 | |
| 250–275 | 27 | 4 | 25 | 3 | 39 | 4 |
| 275–300 | 23 | | 22 | 3 | 32 | 3 |
| 300–325 | 23 | 2 | 16 | 2 | 29 | |
| 325–350 | 20 | 2 | 14 | 3 | 29 | 3 |
| 350–375 | 17 | 1 | 11 | 1 | 22 | 3 |
| 375–400 | 16 | 2 | | | 16 | 1 |
| 400–425 | 12 | | | | 15 | 1 |
| 425–450 | 12 | 2 | | | 10 | 1 |
| 450–475 | 10 | | | | 3 | 1 |
| 475–500 | 6 | | | | | |
| 500–525 | 2 | 1 | | | | |

tumor virus titers, which in turn account for the subsequent enhancement of leukemogenesis. However, since both the additional irradiation and the 6-mercapto-purine treatment initiate or renew immune suppression in previously irradiated mice, the observed inhibition of leukemogenesis must be due to manipulation of a preleukemic change other than immune suppression. This change is radiosensitive. It probably involves critically high numbers of immature, virus-susceptible cells emerging in the hematopoietic tissues recovering from acute radiation injury. Such cells have been directly observed (5, 6) or inferred to exist (7) by various investigators. There is also evidence that high numbers of such cells account for the high incidence of spontaneous thymic lymphoma in certain inbred strains of mice (8). In these strains, administration of exogenous thymolytic agents, such as testosterone or the active adrenal corticoids, lowers the incidence of spontaneous as well as radiogenic lymphoma. Conversely, orchidectomy or adrenalectomy, which results in increased thymic weight, enhances the development of this neoplasm (review Kaplan *et al.*) (9).

Spontaneous, radiation-induced or virologic leukemogenesis may be viewed as quantitative variants of a probabilistic interaction between tumor virus and virus-susceptible cells, comparable to the outbreak of an epidemic in a population containing high numbers of infectious carriers and susceptible individuals (10). This may be postponed or prevented by reducing the number of either carriers or susceptible individuals to a level where a critical interaction is less probable.

It would be interesting to know whether complete prevention of radiogenic murine leukemia can be achieved by reducing the number of virus-susceptible cells. To explore this possibility, postponement should be studied as a function of two variables: the

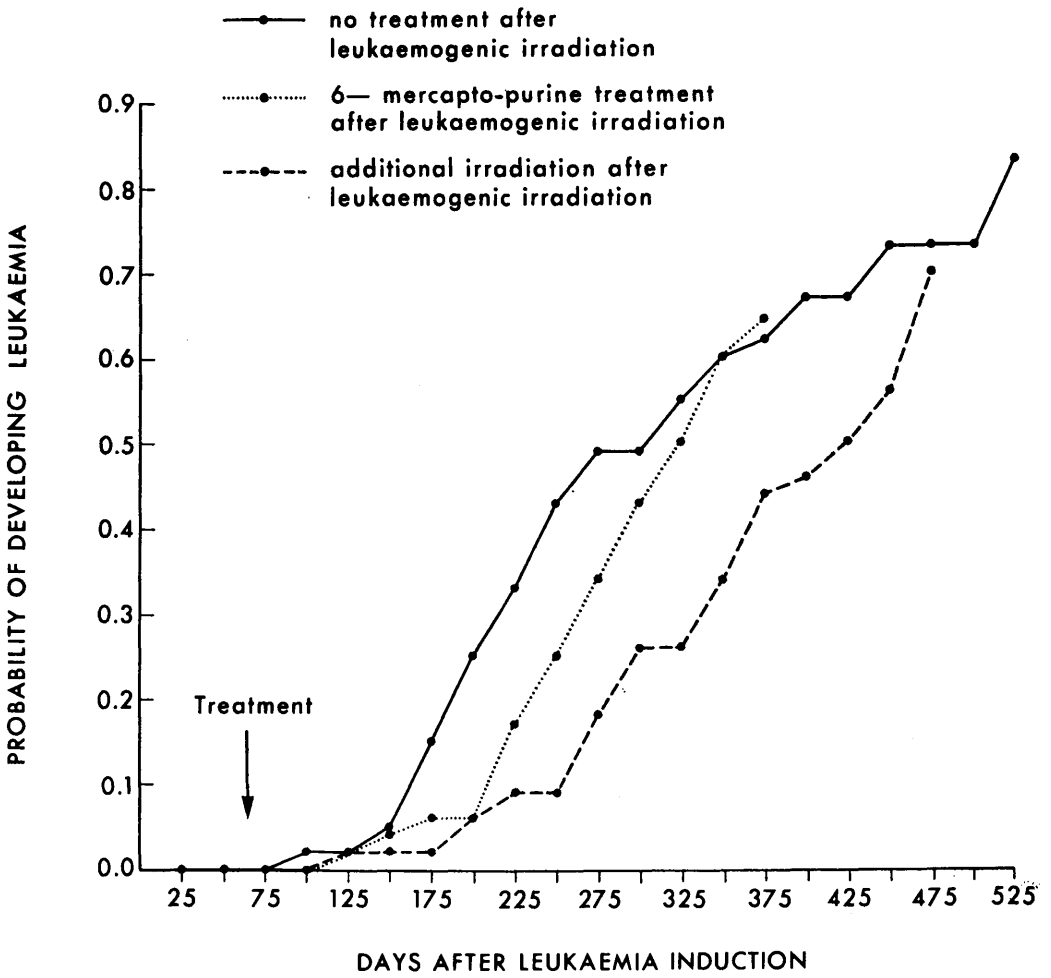


FIG. 1. Postponement of leukemia development by preventive treatment during the preleukemic interval.

radiation dose of the first exposure and the timing of the treatment during the preleukemic interval. Optimum irradiation (in terms of leukemogenesis) may be more difficult to offset by treatment during the preleukemic interval than the conditions created by weaker radiation doses approaching those of spontaneous leukemogenesis. Since the preleukemic changes developing after recovery from acute radiation injury are time-dependent (6) the maximum effectiveness of prevention, too, is likely to be time-dependent.

*Summary.* The same radiation dose which enhances leukemogenesis if delivered to an unirradiated mouse counteracts leukemia development if given to a previously irradiated but not yet leukemic mouse. This indicates that the preleukemic interval between recovery from acute radiation injury and overt malignancy is radiosensitive and that it involves changes other than mere immune suppression. The theoretical optimum conditions of such manipulation are discussed, as well as its implications for the prophylaxis of radiogenic leukemia.

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# Absorption and Distribution of Ethanol during altered Portal Dynamics in the Dog* (33775)

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Many patients with Laennec's cirrhosis present with a long history of alcoholism. Although derangements in portal blood flow often develop during the course of the disease, information concerning the absorption and distribution of alcohol under these circumstances is limited. Therefore, portal blood flow was altered in dogs to simulate abnormalities in patients with hepatic cirrhosis and the absorption of ethyl alcohol and its transport in blood and thoracic duct lymph was studied and compared to control dogs.

*Material and Methods.* Nineteen healthy fasted mongrel dogs (8–22 kg) were studied under light general anesthesia (Myotal). The abdominal aorta, right atrium, and thoracic duct were cannulated with polyethylene tubing in 19 animals, and the portal vein was catheterized after splenectomy in 16 animals. In 12 dogs a single hepatic vein was catheterized via the right external jugular vein, and in three other dogs hepatic venous blood was sampled indirectly from the thoracic inferior vena cava just below a constricting ligature. In 16 dogs the thoracic duct was cannulated in the right chest and ventilation was controlled by a Harvard positive-pressure piston respirator. In three dogs the thoracic duct was cannulated in the neck, respiration was unassisted, and the chest and abdomen were not opened. Blood pressure was followed continuously on a direct writing polygraph (Sanborn) and venous pressures on the polygraph or by saline manometry. After control samples of blood and lymph were taken, and all fluid was carefully aspirated from the stomach, distilled absolute ethyl alcohol (Publiker Industries, Inc.) diluted with distilled water to a 75% solution (3 g/kg) was inserted into the stomach by tube gastrostomy (16 dogs) or through an oro-gastric tube (3 dogs). Simultaneous serial samples of blood and thoracic duct lymph were obtained at specific times after intragastric administration, and alcohol levels were determined in plasma and lymph by titration of free iodine with sodium thiosulfate after diffusion into potassium dichromate and liberation of iodine with excess potassium iodide (1). Lymph from the thoracic duct was allowed to flow by gravity drainage, and, except for small samples taken for determination of alcohol content, was returned to the bloodstream via the right atrial catheter. After 3 hr, gastric contents were aspirated and the remaining alcohol content in the stomach

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