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Inhibition of the development of radiation-induced leukemia in mice by reduction of food intake

(mouse leukemia/caloric restriction/γ-irradiation)

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ABSTRACT **We have reported previously that the incidence of tumors induced in Sprague–Dawley rats by total-body γ-ray irradiation can be considerably reduced by restriction of food intake [Gross, L. & Dreyfuss, Y. (1984) *Proc. Natl. Acad. Sci. USA* 81, 7596–7598]. In experiments reported here we investigated the influence of reduced food intake on the development of radiation-induced leukemia in C3H(f) mice. The incidence of spontaneous leukemia in mice of this strain does not exceed 0.5%, but it can be considerably increased by total-body x-irradiation. In our study, two groups of C3H(f) mice were submitted to fractionated total-body γ-irradiation (150 rads, five times at weekly intervals; 1 rad = 0.01 gray). The first group received a full ad lib diet (4.5–5.4 g of Purina Rodent Lab Chow pellets per day, each). In this group 31 out of 58 females (53.4%) and 24 out of 50 males (48%) developed leukemia at an average age of 8 months. In the second group, consisting of sisters and brothers of the first group, and submitted to the same γ-irradiation but receiving a restricted diet (2 g of Purina Lab Chow pellets each, followed by 3 g on alternate days), only 2 out of 55 females (3.6%), and 1 out of 36 males (2.8%), developed leukemia at an average age of 9 and 12 months, respectively. Leukemia in both groups was predominantly of the lymphatic or lymphoblastic form, the leukemic cells infiltrating most organs, particularly the thymus, mesenteric and peripheral lymph nodes, spleen, liver, kidneys, and bone marrow; in most instances the peripheral blood was also leukemic.**

We have demonstrated in our previous studies that the incidence of radiation-induced tumors in Sprague–Dawley rats can be significantly reduced by restriction of food intake (1). In the study here reported we have investigated the effect of restriction of food intake on the incidence of radiation-induced leukemia in mice. The incidence of spontaneous leukemia in rats is relatively low and is apparently not increased by total-body x-ray irradiation (2). However, it has been known that fractionated total-body x-irradiation of mice of certain strains can induce a high incidence of leukemia and lymphomas (refs. 3 and 4; reviewed in ref. 5). Accordingly, we have set up a series of experiments in which mice of the C3H(f) strain, in which spontaneous leukemia develops only in rare instances (about 0.5%), were submitted to fractionated total-body γ-irradiation. Subsequently, one group of these animals received a full (ad lib) diet of Ralston Purina Rodent Lab Chow pellets, whereas another group received a reduced amount of the same pellet food.

It has been known that the incidence of spontaneous leukemia in Ak mice can be significantly reduced by restriction of caloric intake (6). However, the effect of reduction of food intake on radiation-induced leukemia in mice has not yet been investigated.

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In our previous studies we have demonstrated that radiation-induced leukemia in mice is caused by a transmissible virus, apparently activated by ionizing radiation (7). The filterable virus isolated from radiation-induced leukemia could be passed serially and reproduced leukemia and lymphomas when inoculated into newborn mice (8).

It thus appeared that we had in our hands an excellent animal model for the study of the possible influence of food restriction on the incidence of radiation-induced leukemia. This study was particularly interesting since it referred to radiation-induced leukemia known to be caused by a transmissible virus, whereas in previous studies carried out on rats with radiation-induced tumors, no transmissible virus could be demonstrated.

MATERIALS AND METHODS

Animals. C3H(f) mice, bred in our laboratory for more than 30 years by brother-to-sister mating, were used. These mice are descendants of the Bittner substrain, free from the mammary tumor virus by foster nursing. The incidence of spontaneous leukemia in mice of this strain in our colony does not exceed 0.5%.

Technique of Total-Body γ-Irradiation. Young adult C3H(f) mice received at the Radiotherapy Department of this Medical Center five consecutive total-body γ-irradiations of 150 rads (1 rad = 0.01 gray) each, at weekly intervals. The mice were 3–4 weeks old when they received the first irradiation. The animals were placed in small plastic compartments, 14 cm wide and 24 cm long, each section 9 cm long, 2.5 cm wide, and 4 cm deep, holding one mouse; up to nine mice in nine compartments were irradiated at one time. The technical factors were as follows: Picker cobalt-60 teletherapy unit, 80-cm source-to-midplane distance, portal size of 24 × 14 cm for nine compartments, dose rate of 107 rads/min.

Food Intake. The mice were fed Ralston Purina Rodent Lab Chow 5001. The individual pellets are relatively large; they are rectangular, 2.5–3 cm long, 1.5 cm wide, and 1 cm thick; their approximate weight varies from 3.7 to 5 g.

We have determined in preliminary experiments that mice of the C3H(f) inbred line bred in our laboratory, when allowed to eat as much as they desire, each consume, when 2–3 months old, 3.5–4.3 g of Purina Rodent Lab Chow per day. This amount gradually increases with age, so that a 10-month-old mouse consumes, when allowed to eat a full ad lib diet, an average of up to 5.4 g of pellet food a day.

For mice on restricted diet, we have attempted to determine a reduced food allowance that is relatively small, but nevertheless sufficient to maintain these animals in good health. After several trials we have determined that young C3H(f) mice can be placed, without apparent impairment in their health, on a restricted diet consisting of 2 g of pellet food a day, followed by 3 g of food on alternate days, for each mouse. This amount represents approximately a 36% reduction as compared with young mice on a full ad lib diet. Since the amount consumed increases with age in the ad lib group,

whereas it remains unchanged for mice on the restricted diet, the percentage of the reduction in food intake gradually increases to 46% and even 50% in the restricted diet group.

To conform to our experimental requirements, the Purina Rodent Lab Chow pellets were cut by hand and weighed daily before being fed in proper quantities to those animals that were placed on the restricted diet.

The administration of restricted diet began 21 to 39 days after the last irradiation—i.e., when the mice were approximately 3 months old—and was continued without interruption until the termination of the experiment.

RESULTS

A total of 133 C3H(f) females and 106 C3H(f) males were divided into two groups. In the first group, 65 females and 52 males received γ-irradiation only. They were allowed to eat as much as they desired, and they consumed an average of 3.5–5.4 g of Purina Rodent Lab Chow pellets per day each, the amount of food gradually increasing with age.

In the second group, 68 females and 54 males, sisters and brothers of the first group, received the same γ-irradiation; however, each was placed on a restricted diet of 2 g of the Purina Rodent Lab Chow pellets, followed by 3 g of the same food pellets on alternate days. Both groups also received some lettuce daily. Thus, mice on the restricted diet received an amount of food reduced approximately by 36–50%, as compared with that consumed by their brothers and sisters in the "γ-ray only" group.

All animals in both groups received five consecutive total-body γ-irradiations of 150 rads each at weekly intervals, beginning when they reached 3–4 weeks of age. Up to the age of 3 months, both groups received a full diet. After they reached 3 months of age, the restricted diet group received only a reduced food allowance.

There was considerable mortality among these animals, particularly among males in the restricted diet group, when they were very young. Those that died before they reached 5 months of age, either from intercurrent infections or from cannibalism, but were negative for tumors or leukemia, were eliminated and not included in the tabulation. Accordingly, the totals included in the tabulation (Table 1) were as follows: 58 females and 50 males in the γ-ray only group and 55 females and 36 males in the γ-ray and restricted diet group.

Mice maintained on restricted diet appeared to be very active, at times even hyperactive, in a striking manner, for several hours before feeding time, when they could be observed running upside down, holding on to the wire grids on the top of their cages. Surprisingly, there was not more than at most a 10% reduction in weight in some of the mice on restricted diet, as compared with those allowed to eat ad lib.

At the time of this writing, among the 58 irradiated females on a full ad lib diet, 31 (53.4%) developed leukemia at ages varying from 5.5 to 11.5 months (average 8 months). Among 50 males in this group, 24 (48%) developed leukemia at ages varying from 6 to 11.5 months (average 8 months).

In contrast, in the γ-ray and diet group, only 2 out of 55 females (3.6%) and 1 out of 36 males (2.8%) developed leukemia; the females developed leukemia at the ages of 8 and 10 months, respectively; the male developed leukemia at the age of 12 months (Table 1).

In most instances leukemia developing in the irradiated mice was generalized, with involvement of peripheral and mesenteric lymph nodes, and replacement of thymus by a lymphoid tumor. Occasionally, the thymic lymphoma was very large, filling out most of the chest cavity, with relatively minor involvement of other organs.

On microscopic examination, leukemic infiltrations were found in several organs; the spleen, lymph nodes, and bone marrow were most commonly involved. In a substantial number of animals, liver and kidneys were also affected; a predilection for hepatic periportal tracts and renal cortex was noticed. In all the organs, the extent of the leukemic infiltrates varied widely, from patchy microscopic foci to massive invasion of the parenchyma. Cytologically, the leukemic cells were consistently highly undifferentiated; when signs of maturation were present, they seemed to indicate a lymphocytic form. The majority of the induced leukemias were of the lymphatic or lymphoblastic form. In about 80% of leukemic mice, blast cells were present in peripheral blood.

Electron microscopic examination of a typical leukemic lymph node revealed C type virus particles (Fig. 1).

The remaining animals in both groups, which are still alive and apparently in good health, are now 9–14 months old. It is anticipated that additional irradiated mice will develop leukemia in both groups. Even at the present time, however, the trend is clear. The difference in the numbers of animals that developed leukemia in the group on a full ad lib diet, as compared with those on restricted food intake, as well as the apparent difference in the latency of the onset of the disease, are striking and warrant a preliminary communication.

DISCUSSION

We have reported previously that the incidence of tumors induced in Sprague–Dawley rats by total-body γ-irradiation could be considerably reduced by restriction of food intake (1). In the study here reported we have examined the possibility of a similar effect of reduction of food intake on the development of radiation-induced leukemia. This study was carried out on mice. Total-body x-irradiation substantially increases the incidence of tumors, but not leukemia, in rats (2). On the other hand, total-body x-irradiation of mice of certain inbred lines, such as C3H, C3H(f), or C57BL, leads to a significant increase in the incidence of leukemia and lymphomas, but only very occasionally in that of tumors

Table 1. Effect of restricted diet on the incidence of leukemia in γ-irradiated mice

			Mice developing leukemia					Mice that died negative				Surviving well mice			
					Age when leukemia developed, mo				Age when died, mo				Age, mo		
Treatment*	Sex	No. mice obs	No.	%	Min	Max	Avg	No.	Min	Max	Avg	No.	Min	Max	Avg
γ-ray only	F	58	31	53.4	5.5	11.5	8	3	8	12	9.7	24	9	14	10.7
	M	50	24	48	6	11.5	8	3	8.5	12	10.3	23	9	14	10.7
γ-ray and restricted diet	F	55	2	3.6	8	10	9	12	5	13	8.9	41	9	14	11.4
	M	36	1	2.8	12		12	16	5	13	7.2	19	9	14	10.8

F, female; M, male.

*Five consecutive total-body γ-irradiations, 150 rads each, at weekly intervals. Age of mice at first irradiation was 3–4 weeks. Each mouse on restricted diet received 2 g of Purina Rodent Lab Chow pellets a day, followed by 3 g on alternate days, as compared with amounts varying from 3.5 to 5.4 g, increasing gradually with age, consumed by mice on a full ad lib diet.

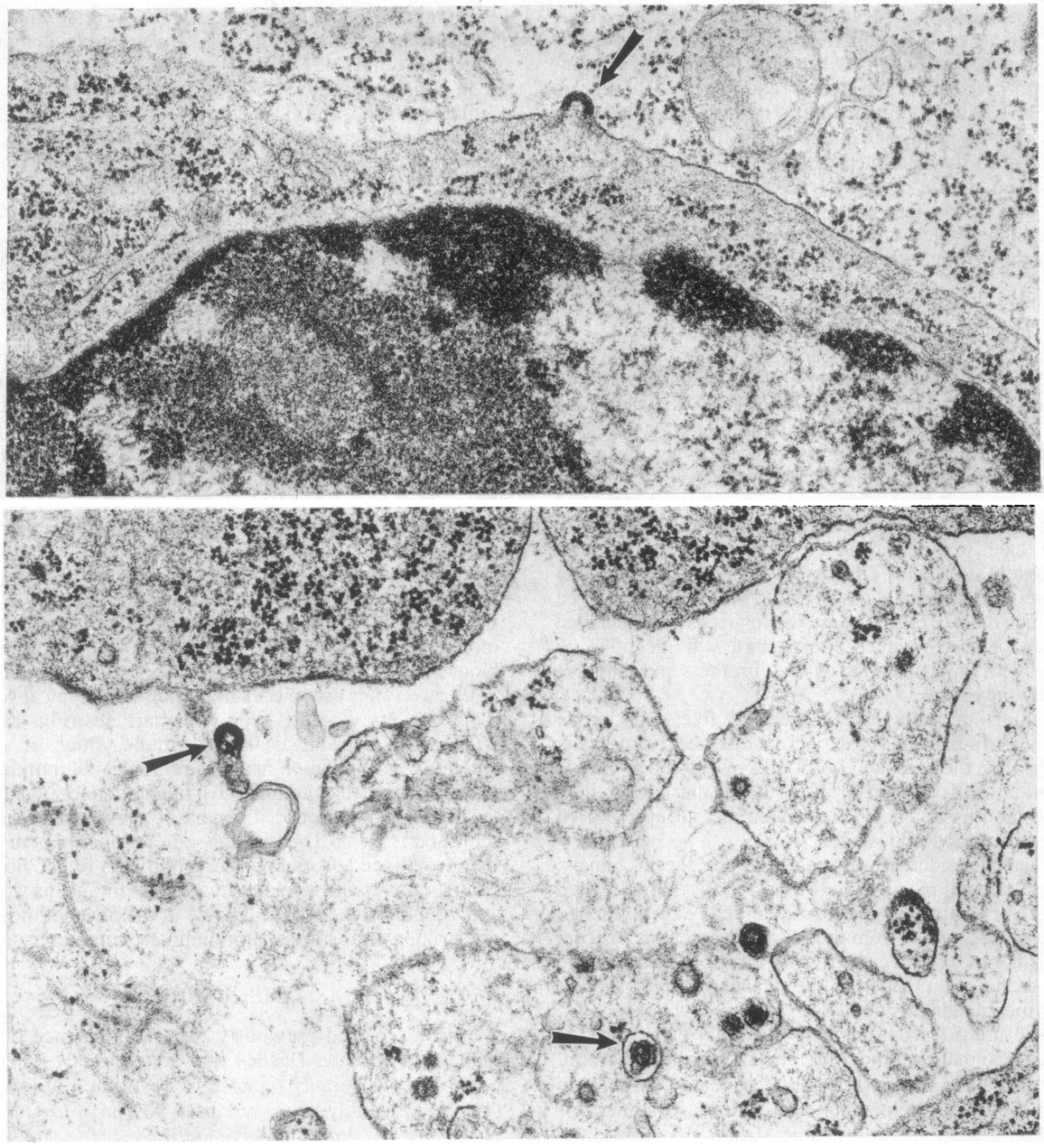

FIG. 1. Part of a cell from a leukemic lymph node removed from a C3H(f) mouse that developed leukemia after five consecutive total-body γ-irradiations. Note budding (arrow in *Upper* and left arrow in *Lower*) and mature (right arrow in *Lower*) C type virus particles. The mature virus particles are spherical and have a diameter of approximately 100 nm, an electron-dense nucleoid, and an outer shell. The mature virus particle shown (*Lower*, right arrow) is located in a vacuole. (×40,000.)

(3–5). It should be emphasized that, with rare exceptions, the same animals remain free from leukemia when untreated and allowed to live their life spans undisturbed under normal laboratory conditions; in our colonies of C3H and C3H(f) mice the incidence of spontaneous leukemia does not exceed 0.5%.

In our previous experiments, in which we used C3H or C3H(f) mice for fractionated total-body x-irradiation, 55 out of 116 (48%) irradiated mice developed leukemia, as compared with none (0%) among 93 nonirradiated littermate controls (4). From organs of C3H(f) mice that developed leukemia after total-body x-irradiation, we were able to isolate a virus that could be passed serially, inducing leukemia after inoculation into newborn mice (7, 8). A similar observation was made independently on mice of the C57BL inbred line (9). It is apparent therefore, that mice of certain strains carry a latent virus that may, when activated, induce leukemia. One of such activating factors is γ-irradiation. Dorothy Feldman observed in our laboratory that virus particles may appear in fragments of cells of the spleen, bone marrow, and lymph nodes, occasionally in relatively large numbers, within a few days, in some instances as soon as one day, after total-body x-irradiation of C3H(f) mice, although leukemia usually develops in the irradiated animals only 6–8 months later. Some of the most striking electron micrographs showed formation of virus particles budding from several areas along the cell membranes of plasma cells in the spleen, 15 days after irradiation (10).

The fact that reduction of food intake may significantly decrease the incidence of leukemia, and therefore presumably prevent virus activation, is of considerable interest and may have important implications. We theorize that activation of an oncogenic virus may be readily prevented, and that restriction of food intake may represent only one of such preventive measures.

Inhibition of radiation-induced leukemia in mice by restriction of food intake is consistent with statistical observations suggesting a higher incidence of malignant tumors in both men and women who are substantially overweight (11). In our study, however, mice on a full ad lib diet had only at most a slightly higher average weight than those on restricted diet.

In conclusion, if our observations on mice could be applied to humans, it would follow that moderation in food intake should be considered as one of the recommended measures in attempts to prevent the development of leukemia, lymphomas, or other malignant tumors, resulting from exposure to ionizing radiation.

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