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Experimental Manipulation of Preleukemic Change in Whole-Body Irradiated RFM/Up Mice[1] (38542)

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(Introduced by A. C. Upton)

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Radiogenic leukemia is preceded by a preleukemic state physiopathologically distinct from the overt disease and involving two major component processes. The first governs the immunologic equilibrium between tumor virus and host, the second the tumor–virus susceptible "target cells".

The relationship between both may be described by the following model. If the immunologic equilibrium between tumor virus and host is disturbed by an immune suppressant, such as ionizing radiation, the tumor virus titer of the host tissues may increase (5, 11, 18). Conversely, if cell losses in the hematopoietic or lymphoreticular tissues elicit regenerative responses or if the normal maturation of cell lines is impaired, the number of immature, virus-susceptible cells may rise, thus increasing the chance of an oncogenic response to virus (6, 9). The incidence of leukemia in a population or the probability of leukemia in an individual increase with any condition resulting in a rise of the number of virus particles or of the virus susceptible target cells, or of both (12).

This model readily explains the following observation. The same radiation dose which enhances leukemogenesis if delivered to an unirradiated mouse, reduces leukemia development if given to a previously irradiated, but not yet leukemic mouse (14, 17). If immune suppression were the only component of the preleukemic state, the second irradiation would be expected to enhance the leukemogenic effect of the first. The postponement of leukemia by the second irradiation, however, is consistent with the assumption that the preleukemic state entails, besides impairment of antibody production, the emergence of cell populations responsive to both virus and radiation.

The experiment reported here was designed to test this model further. The specific question to be answered was twofold: (1) How is the known late radiation injury of the bone marrow modified by additional irradiation resulting in postponement of leukemia? (2) Does this modification throw further light on the pathogenesis of radiogenic leukemia?

Materials and Methods. Animals. Forty-eight male RFM/Up mice were used. The initial stock was supplied by the Biology Division of the Oak Ridge National Laboratory and inbred for this project by Simonsen Laboratories, Gilroy, CA. The mice were maintained with Purina Chow and tap water *ad libitum*.

Basic design. Thirty-four mice received a leukemogenic exposure to X-rays when they were 60–90 days old. Half of them were reirradiated under identical conditions 60 days later. Fourteen other mice were used as unirradiated controls. Out of each of these three groups, two mice were sacrificed 48 hr after the first irradiation. Forty-eight hours after the second irradiation, the following numbers of mice were sacrificed: Four from the group having received two irradiations, four from the group having received one irradiation, and two from the nonirradiated control group. The remaining population, which comprised 10 unirradiated control mice and 11 mice in each irradiated group, was sacrificed 60 days after the second (or 120 days after the first) irradiation. The experimental design thus follows that which originally established the postponement of radiogenic leukemia by reirradiation (14), except that radiation injury is studied in sequentially sacrificed animals instead of leukemia incidence in animals allowed to live out their lifespan.

Since the total number of mice required for the experiment could not be made avail-

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able simultaneously at the beginning of the experiment, animals were randomly assigned to the three experimental groups at a rate determined by the output of the breeding colony. This took about 6 wk.

Radiation factors. A General Electric Maxitron was used. Both irradiations, whether used for the purpose of leukemia induction or postponement, involved exposure to 300 rads of 250 kV X-rays. Half value layer 1.5 mm Cu; absorbed dose rate in soft tissue 27 rads/min full scatter; target distance 70 cm. During exposure, the mice were placed in a wooden box of 30 × 30 cm, covered with a translucent plastic top leaving a depth of 2 cm. The mice were allowed to move freely within the field.

Endpoint observed. The tissues of each animal were fixed in Bouin's solution embedded in paraffin, sectioned at 4 μm, and stained with hematoxylineosin. The following organs were histologically examined: Thymus; spleen; liver; axillary, inguinal and retroperitonecal lymph nodes; left lung; left kidney; brain; left adrenal; a longitudinal section of the right decalcified femur.

The absolute numbers of various cell types of the marrow of the left femur were determined for each animal sacrificed. The shaft of the femur was split and the marrow content was scraped into 1 ml of heparinized porcine serum containing 10 × 10^6 basidiospores of Calvatia gigantea (7). Thin films, containing not more than five to ten particles per oil immersion field, were made. The ratio cells over spores was determined in the film and the number of cells present in the suspension calculated. A complete description of this method has been published elsewhere (13). One set of two slides was stained by means of the indophenol blue technique, and another set of two slides stained by hematoxylin and eosin alone. In the set stained by means of the indophenol-blue technique, red blood cells and "mature myeloid cells" were counted. The latter include mature myelocytes, metamyelocytes, neutrophiles, and eosinophils, i. e., the cells containing enzymes capable of catalyzing the condensation of alpha-naphtol and paraphenylendiamin. In the set stained by hematoxylin eosin alone, lymphocytes, erythroblasts (nucleated cells of the red series except promegaloblasts) and "precursor cells" were counted. In any given sample, the number of the precursor cells was obtained by subtracting the count of the mature myeloid cells from that of the entire myeloid series. The percentage of the mature myeloid cells in the entire myeloid lines provided an estimate of the marrows maturity. This percentage will be referred to as the "myeloid index."

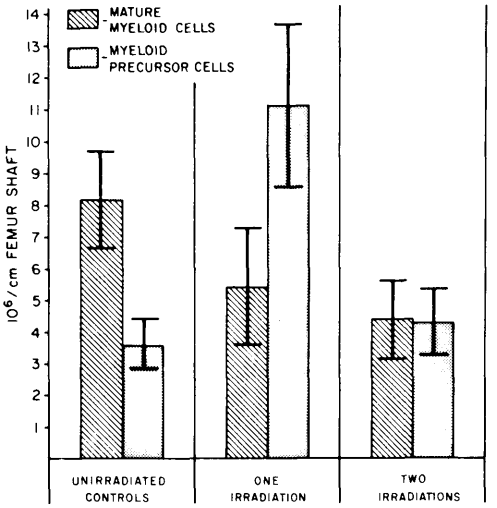

FIG. 1. Modification of late radiation injury of the femcral myeloid system by additional irradiation. Each pair of columns represents the mean cell counts (left femur) of 10 or 11 mice ±1 SD. At the time of sacrifice, the mice were 180–210 days old. The mice represented by the center columns received one leukemogenic irradiation 120 days earlier. In the mice represented by the right columns, this irradiation was followed by an identical irradiation 60 days later.

Results. No mouse died spontaneously prior to the scheduled sacrifice, nor did the histological examinations after sacrifice reveal tumors.

Both irradiations resulted in acute radiation damage. In the bone marrow of the mice sacrificed 48 hr after irradiation, a drop of the number of the nucleated cells and a rise of the number of the erythrocytes was observable. In the mice receiving two irradiations, no difference between the acute response after the first and the second irradiation was observable, except that the rise of the marrow erythrocytes was significantly higher after the second irradiation.

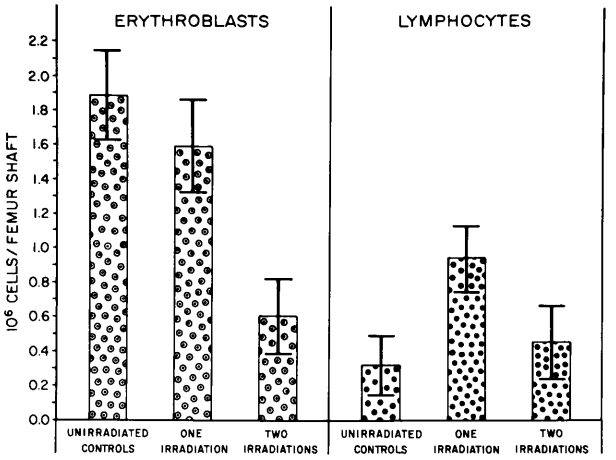

FIG. 2. Modification of late radiation injury of the femoral erythroblasts and lymphocytes by additional irradiation. Each set of three columns represents the same three groups of mice shown in Fig. 1.

The data on the late effects are set out in Figs. 1 and 2. In the femoral marrow of all mice sacrificed 120 days after the first or 60 days after the second irradiation, nonmalignant late effects are recognizable. The length (10 mm) and the weight (34.5 mg) of the femur shaft, however, were the same in the controls and the two irradiated groups. Likewise, the number of the marrow erythrocytes (1.83×10^6/cm femur shaft) was the same in the three groups of mice.

Figure 1 summarizes the data on the myeloid cell count of the femoral marrow. In the unirradiated mice, 8.2×10^6 mature (indophenolblue positive) granulocytes were found per cm femur shaft, 3.6×10^6 was the corresponding value for the myeloid precursor forms. 69.5% of all cells belonging to the myeloid series were thus mature, indophenolblue-positive forms. In the animals irradiated under optimum conditions of leukemia induction, the number of the mature end forms had dropped to 5.4×10^6 cells/cm femur shaft, that of the myeloid precursor forms had risen to 11.11×10^6 cells. Both departures from the control levels are significant ($P < 5\%$). The percentage of the mature cells in the entire series was only 32.7% in this group. Reirradiation alters this late effect of the earlier radiation exposure. The mature myeloid cells have now fallen to 4.4×10^6. This value is not significantly different from that found in the mice having received one single irradiation. The myeloid precursor forms, however, which had reached very high levels in the group having received one single exposure, has now dropped to 4.3×10^6 cells. This value is quite close to that of the unirradiated controls. The difference between the reirradiated group and that having received one single exposure is highly significant ($P < 1\%$). In the reirradiated mice, the percentage of the mature myeloid cells in the entire series is 50.5%.

Table II summarizes the data on the erythroblasts and the lymphoid cells of the femoral marrow. The unirradiated controls showed 1.88×10^6 erythroblasts per cm femur shaft, the mice having received one single radiation exposure 1.59×10^6, the reirradiated mice 0.6×10^6. The difference between controls and the animals having received two irradiations is significant ($P < 1\%$), but not the difference between controls and the mice having received one irradiation only. The data are nonetheless consistent with earlier reports on permanent damage in the erythropoietic cell system in whole-body irradiated mice (1). The late effects seen in the marrow lymphocytes resemble those in the myeloid series after recovery from acute injury. The leukemogenically irradiated mice show a significant rise of their lymphocyte levels over that of the controls (0.94 vs 0.33×10^6 cm femur shaft, ($P < 5\%$)). Reirradiation, at the time the mice were sacrificed, has brought this

increase back to the control level (0.44×10^6).

Discussion. Leukemia incidence appears to be positively correlated with the absolute number of the myeloid precursor cells and of the marrow lymphocytes, and negatively correlated with the "myeloid index" (the percentage of the mature myeloid cells in the entire myeloid series). It has been reported previously that whole-body irradiation, once recovery from the acute depletion of cells is completed, may result in lymph nodes larger than the normal (8, 3) and a hypercellular and less mature bone marrow (4, 16). In the RFM/Up strain at least, the radiation factors and the age at exposure required to bring about this late effect are identical to those of optimum leukemia induction (16). Conversely, the additional irradiation employed in the present experiment offsets this late effect of radiation in the blood forming tissues. It is known from previous experiments that such reirradiaton postpones significantly the development of leukemia (14).

The hematologic late effect, the modification of which may account for the postponement of leukemia seen in earlier experiments has thus two distinct but closely related facets: the high number of immature cell types and the low myeloid index. Which is critical in terms of leukemia induction? An answer to this question is afforded by considering the following previously published data. In mouse strains used in leukemia research, both spleenshielding during irradiation and splenectomy prior to irtadiation reduce leukemia incidence by about 30% (10, 15, 21); but spleenshielding, curiously enough, boosts the overregeneration of the myeloid precursor cells whereas splenectomy prior to irradiation prevents it from occurring (15). However, as a result of both experimental conditions the myeloid index reverts to the level of the unirradiated controls. Thus, meaningful correlations between leukemia incidence and hematopoietic late effects of radiation can be established only by considering the myeloid index, not absolute cell numbers (15). The available data indicate that this index, irrespective of the cell numbers involved, is correlated with leukemia incidence, whether this incidence varies as a function of the strain (16), the radiation dose (15), removal or shielding of a hemopoietic organ, or, as in the present experiment, of an additional irradiation.

How can this correlation be explained in terms of the model presented in the introduction of this report? As has been pointed out, fundamental to our understanding of preleukemic change is whether susceptibility to tumor virus is a constant, inherent characteristic of immature hematopoietic cells, or whether this susceptibility can be acquired, enhanced or cancelled by influences from the cells environment (12). The result of this experiment suggests the latter. The picture is consistent with the assumption that removal of a controlling influence, exerted by the mature cells over their precursors, accounts for the proneness of the irradiated mice to develop leukemias. This controlling influence, in the light of current concepts, may be feedback inhibition of cell replication (2, 19, 20), the loss of which results in overregeneration and vulnerability to viral oncogenesis. It would seem of interest to study this in a variety of histosystems involving sequential differentiation of cells.

Summary. Radiation which induces leukemogenesis in the unirradiated mouse, inhibits leukemogenesis if given again to the previously irradiated, but not yet leukemic mouse. The objective of this experiment was to identify nonmalignant late radiation injury in the bone marrow, the modification of which by a second exposure may account for postponement of radiogenic leukemia. To this end, leukemogenically irradiated RFM/Up mice were reirradiated under conditions known to bring about postponement of the leukemias due to the first radiation exposure. Quantitative analysis of the femoral bone marrow of these mice shows that leukemia incidence is positively correlated with the number of immature myeloid cells, and negatively correlated with the percentage of the mature myeloid cells in the entire myeloid series. The implications of this for the understanding of preleukemic change are discussed.

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