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Late Somatic Effects in Mice after Total Lymphoid Irradiation

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Late somatic effects of total lymphoid irradiation have been investigated in $BC3F_1$ mice. A total X-ray dose of 34 Gy was distributed in 17 daily fractions. The cumulative mortality curve is shifted in time because all the irradiated mice died earlier than the unirradiated controls. There was a 24% shortening of life span. A marked increase of solid tumor incidence, mostly due to skin cancers, was observed (66% vs 30%). In contrast, the incidence of malignant lymphomas was greatly reduced in irradiated mice (6% vs 49%). Furthermore, nephrosclerosis was a common finding in the irradiated group (38% vs 8%). Death-rate analysis revealed an association between life shortening and the presence of solid tumors and nephrosclerosis at death.

INTRODUCTION

The immunosuppressive effects of total lymphoid irradiation (TLI) have been extensively investigated in experimental animals (*1–6*). Among the various factors contributing to immunosuppression, it has been shown that TLI decreases helper T cell activity, increases suppressor T cell activity, and impairs thymic functions. Since these mechanisms play a role in the growth of some tumor types (*7, 8*), TLI might be expected to increase the risk of malignancy.

Long-term effects of TLI, however, have not yet been described in experimental animals. Thus the present study was undertaken in mice with a high incidence of spontaneous lymphomas to investigate whether exposure to TLI (1) decreases the expected frequency of this type of neoplasm at death, (2) is associated with secondary malignancies, and (3) induces a shortening of life span.

MATERIALS AND METHODS

Animals and irradiation procedure. The animals used were adult (6-month-old) male hybrid mice (hereafter referred to as $BC3F_1$) resulting from crossing C57B1/Cne females and C3H/HeCne males. They were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL) given ip at a dose of 65 mg/kg body wt, and positioned in an apparatus similar to that described by Slavin *et al.* (*9*). The apparatus is designed

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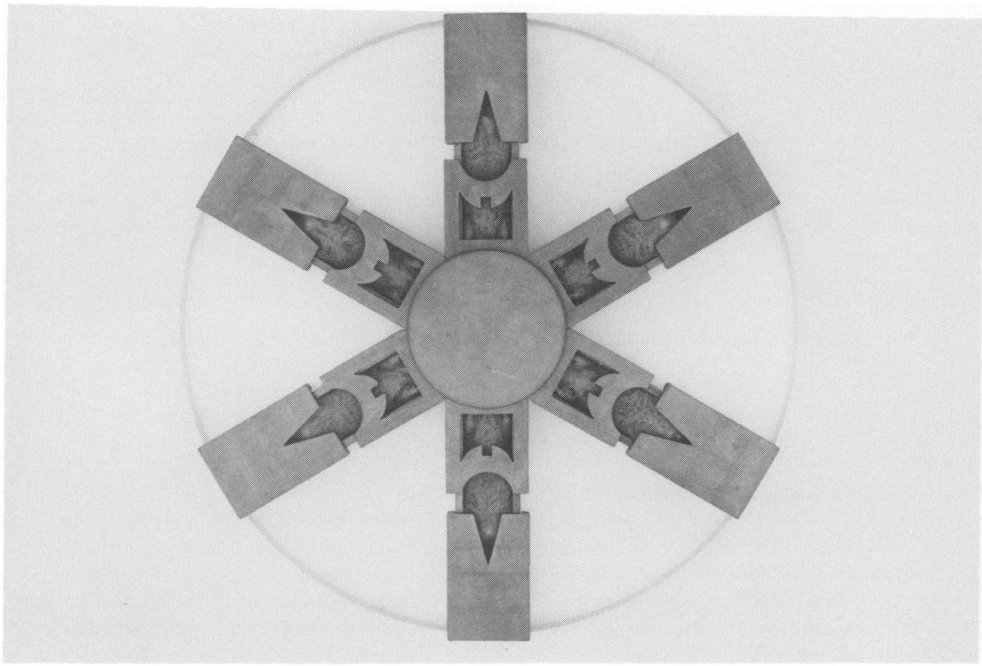

FIG. 1. Lead container with six anesthetized mice as used for total lymphoid irradiation (TLI).

to expose the major lymphoid organs to X rays (Fig. 1). It consists of six lead tubes, with rectangular cross sections (inner dimensions 2.5 × 2 cm, wall thickness 4 mm), placed radially. The upper side of each tube is made up of two portions free to slide horizontally and shaped in such a way as to provide appropriate protection for the parts of the mouse body to be spared. An additional centrally-placed lead disk completes the shielding. The animals received 17 daily doses (from Monday to Friday for three consecutive weeks and on Monday and Tuesday of the fourth week) of 2 Gy of X rays from a deep therapy unit operated at 250 kVp, 15 mA, HVL = 0.2 mm Cu, dose rate 1.25 Gy/min, 50 cm distance from the focus, with the vertical beam oriented in the ventral–dorsal direction. The dose distribution was measured along the axis of a cylindrically symmetric mouse phantom using high spatial resolution thermoluminescent dosimeters (*10*). The results are shown in Fig. 2 and prove the effectiveness of shielding. A total of 118 mice were irradiated; 68% of these survived more than 1 month and were entered in the long-term follow-up. Six of

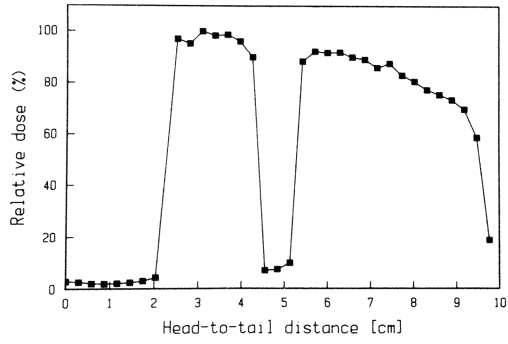

FIG. 2. Dose distribution along the axis of a cylindrically symmetric mouse phantom shielded as for TLI.

TABLE I

Survival and Incidence of Neoplastic and Nonneoplastic Diseases at Spontaneous Death in $BC3F_1$ Mice after TLI

	No. of mice	Mean survival time ± SD (days)	Nephro-sclerosis	Malignant lymphoma[a]	Solid tumors					
					Lung	Liver	Skin	Soft tissues	Others[b]	Total
Control	88	887 ± 189	7 (8)	43 (49)	5 (6)	12 (14)	1 (1)	4 (5)	4 (5)	26 (30)
Irradiated	68	673 ± 144*	26 (38)*	4 (6)	3 (4)	13 (19)	18 (26)*	5 (7)	6 (9)	45 (66)*

Note. Numbers in parentheses are percentages.

[a] FCC-lymphoma, mixed cell type.

[b] Adrenal, kidney, stomach, bone, and vascular tumors.

* $P < 0.001$ in the Mann–Whitney U test for the comparison between survival times and in the χ^2 test for the comparison between frequencies.

these animals, chosen at random, were sacrificed at 37 and 76 days after the last dose for the determination of the colony-forming unit (CFU) content of the spleen and the shaft of the humerus. Ninety-seven age-matched controls were anesthetized and sham irradiated. Five of these animals were used for CFU titration.

Colony-forming unit assay. The hemopoietic stem cells in the spleen and in the humerus shaft were assessed by the spleen colony test of Till and McCulloch (*11*). The procedures applied to bone marrow and spleen cell preparations have been reported elsewhere (*12*).

Follow-up and pathology. All mice were inspected daily (6 days per week) for their entire life span. Soon after spontaneous death, a complete autopsy was performed on 68 (92%) of the 74 irradiated mice and on 88 (96%) of the 92 unirradiated mice. Tissue masses, as well as sections of major organs, were taken and processed for histological analysis. Tissues were fixed in Bouin's fluid and processed for paraffin embedding and sectioning. Sections were stained with hematoxylin and eosin. The histological diagnoses were coded and entered in a computer for statistical analysis.

Analysis of cumulative mortality. The Weibull distribution (*13*) was used to model the mortality distribution with time for all causes of death of the mice which survived at least 1 month after irradiation. This model is a generalization of the exponential distribution, as it does not assume a constant hazard rate, and therefore has broad applicability. The cumulative mortality $M(t)$ as a function of the time t is expressed as

$$M(t) = 1 - [\exp - (\lambda t)^{\gamma}], \tag{1}$$

where γ and λ are the shape and scale parameters, respectively. The maximum likelihood estimates of γ and λ were obtained for each group by an iterative procedure. A two-sample test, proposed by Thoman and Bain (*13*) for samples without censoring, was used to compare the resulting fitted mortality distributions.

Analysis of specific death rates. Age-related death rates for specific lesion types, diagnosed at death, were computed and plotted as cumulative probabilities as a function of time, according to the model of Rosenblatt *et al.* (*14*). In essence, this method analyzes both the frequency of a disease at death and the observation time by a single set of statistics, taking competing risks and losses into account, and is useful for the comparison of different experimental groups (*15*).

RESULTS

The mean life spans of the irradiated and the control mice are shown in Table I. The life span of the irradiated group is significantly shorter (Mann–Whitney U test, $Z = -7.34$, $P < 0.001$) than that of the control group, with a maximum difference of approximately 200 days (24%). The corresponding curves of cumulative mortality for all causes were well fitted according to Eq. (1) ($\gamma = 6.659$, $\lambda = 1.385 \times 10^{-3}$, $P = 0.92$ for the irradiated group, and $\gamma = 5.719$, $\lambda = 1.041 \times 10^{-3}$, $P = 0.93$ for the

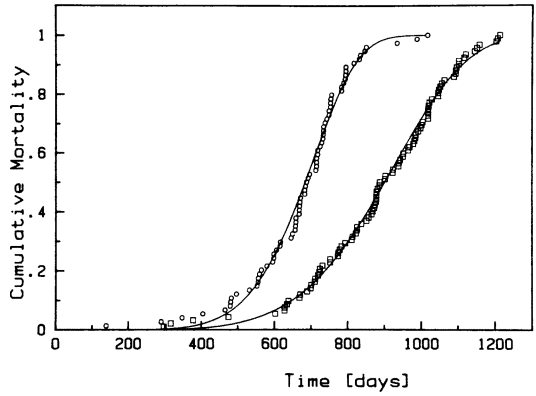

FIG. 3. Curves of cumulative mortality for unirradiated (□) and TLI-treated (○) mice. Curves were fitted with Eq. (1).

control group) and were found to be significantly different (Thoman and Bain test, $G = 1.767$, $P < 0.02$). As shown in Fig. 3, the cumulative mortality curve of the TLI-treated mice shows a forward shift in time compared to that of the control group.

Table I also shows the incidence of degenerative diseases, lymphoid neoplasms, and solid tumors. The frequency of nephrosclerosis was higher in the irradiated group than in control mice ($P < 0.001$). On macroscopic examination the kidneys were reduced in size and showed irregular scarring of the cortex. Histological observation revealed damage involving the glomeruli with a diffuse degree of hyalinization. This lesion was often accompanied by interstitial chronic inflammation.

The incidence of malignant lymphomas was greatly reduced in the irradiated mice compared to unirradiated mice ($P < 0.001$). According to Pattengale and Frith's classification (*16*), based on anatomical location and cytomorphology, all the lymphomas observed in the present study were of the B-cell type, mixed follicular center-cell lymphomas (FCC-lymphomas).

In contrast, the frequency of solid tumors was found to be significantly increased in the irradiated animals with respect to the control group ($P < 0.001$), mostly due to squamous cell carcinomas of the skin. These carcinomas were localized in the skin of the ventral side of the mouse, especially on the abdomen and chest. Morphologically, they were composed of highly undifferentiated cells which had invaded the subcutaneous tissues. In eight cases, the presence of metastases was histologically confirmed in the lungs and the lymph nodes.

These findings are consistent with the results of the analysis of age-related death rates for specific causes. In particular, as shown in Fig. 4, the nephrosclerosis (A) and solid tumors (B) start earlier and develop faster in the irradiated animals. The risk of death from these two causes reaches its maximum at about 700 days of age, while in the control mice the risk is lower and remains more or less constant later in life.

Table II shows that TLI negatively affects the CFU frequencies in bone marrow and spleen. It appears that the CFUs in the irradiated mice, observed at 37 and 76 days after the end of the TLI treatment, are significantly less frequent than in the unirradiated control group.

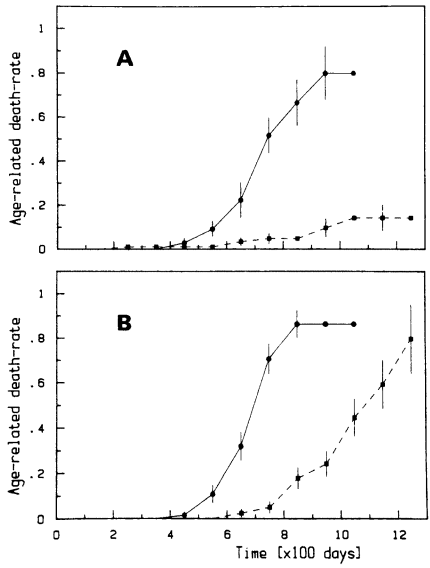

FIG. 4. Curves of age-related death rates for nephrosclerosis (A) and solid tumors (B) in unirradiated (■) and TLI-treated (●) mice. Bars are standard errors.

DISCUSSION

In the present experiment, about two-thirds of the irradiated mice survived the first month after irradiation. The acute mortality observed is similar to data previously reported in mice (*1*).

The irradiated animals developed a significantly higher number of solid tumors, compared to those of the control group, mainly because of the increased incidence of primary skin cancers.

The preferential localization of skin cancers on the ventral side of the mouse may be the result of the absorption of a higher radiation dose by the ventral skin in comparison to the dorsal skin. However, since the difference in absorbed dose between the two sides was calculated at only 20% (10), this cannot be the unique factor which accounts for the localization of the skin tumors. It has also been reported by Fry *et al.* (*17*) that tumors are induced in the mouse skin by local irradiation with soft X rays (25 kV), after high fractionated doses, only when the promoting agent 12-*O*-

TABLE II

CFU Content in Bone Marrow (BM) and Spleen (S)

		TLI-treated mice at	
	Control mice	*37 days*	*76 days*
CFU/10^4 BM cells	3.32 ± 0.21	0.63 ± 0.07*	1.50 ± 0.08*
CFU/10^5 S cells	1.60 ± 0.19	0.84 ± 0.09**	0.50 ± 0.06*

Note. Quoted values are means ± SE.

* $P < 0.001$ and ** $P < 0.01$ in the Student's t test.

tetradeconyl phorbol-13 acetate is applied to the exposed region for several weeks after irradiation, and the dose–effect relationship is a threshold-type one. In this respect, it is conceivable that TLI has a tumor promoting effect in that it strongly depresses the immune system. It is, indeed, possible that TLI favors the development of suppressor T cells (*3, 5, 6*) which may play a role here similar to that in the induction of skin tumors in ultraviolet-irradiated mice (*18, 19*).

Previous reports have indicated that the rodent kidney is sensitive to the development of radiation-induced degenerative lesions after exposure to high single acute doses (*20, 21*). In the present experiment, nephrosclerosis is the only nonneoplastic disease observed at death, and its frequency is remarkably higher than in the unirradiated animals. Therefore, the fractionation scheme used in this study does not appear to spare the kidneys.

As already reported by UNSCEAR (*22*), the incidence of malignant lymphomas is high in unirradiated $BC3F_1$ control mice (about 50%) and is very effectively depressed to a low incidence after total-body exposure to high doses of X rays, regardless of the time distribution of the dose (acute or fractionated). TLI also causes a remarkable decrease in the frequency of malignant lymphomas. This effect may be attributed to the killing of the stem cells of the hemopoietic system susceptible to neoplastic transformation, which is supported by the marked and long-lasting reduction of the CFU frequencies in bone marrow and spleen of TLI-treated mice (Table II). The existence of a positive correlation between the incidence of malignant lymphomas and the number of hemopoietic stem cells in $BC3F_1$ mice has also been suggested by the results of previous studies on long-lived syngeneic chimeras (*23, 24*). The profound and long-lasting reduction of CFUs in hemopoietic tissues (*25*) might also account for the life shortening in TLI-treated mice by as yet undefined mechanisms. Furthermore, analysis of the mortality also indicates that the presence of solid tumors and/or degenerative diseases may play a role in causing life shortening, although it is difficult to ascertain the relative importance of each class of disease. As far as malignant lymphomas are concerned, the rate at which they appear during the lifetime of unirradiated mice is similar to that of the cumulative mortality for all causes in the same group. Therefore, the TLI-induced depression of malignant lymphomas cannot substantially affect the longevity of the irradiated mice.

The possibility of extending the conclusions from this type of experimental study to clinical applications is questionable due to existing differences in TLI procedures and in species radiosensitivity (*26*). The differences in tumor incidence between irradiated and unirradiated mice we found were statistically highly significant for both the total solid tumors ($P < 0.001$) and the primary skin cancers alone ($P < 0.001$). Therefore, we suggest that the risk of inducing late effects by TLI in humans cannot be excluded a priori and therefore should be carefully considered.

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