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Effect of Intravenously Injected Bone Marrow Cell Suspensions on Thymic Regeneration in Irradiated C 57 Black Mice[1]

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The development of thymic lymphomas in systemically irradiated C 57 black mice is effectively inhibited by placing a lead shield over one thigh during exposure, despite the fact that the thymus receives the same x-ray dose (*1, 2*). It was recently shown that, although initial radiation injury to the thymus is not prevented, thymic regeneration is significantly accelerated by thigh shielding (*3*). This report is concerned with a series of four experiments indicating that this effect of thigh shielding may be largely reproduced by intravenous injection of homologous bone marrow cell suspensions into systemically irradiated mice. Regeneration of the thymus begins less promptly, however, and requires the injection of relatively large amounts of bone marrow. The intraperitoneal implantation of skeletal muscle and the intravenous injection of exogenous thymic cell suspensions were not effective in promoting thymic recovery.

In each of the four experiments, littermate C 57 black mice of both sexes, aged 33 ± 3 days at the start of treatment, were distributed equally among 3–6 groups. These groups included both untreated and irradiated controls and four types of experimental groups. The irradiated controls received an intravenous placebo injection of Locke's solution, 0.05–0.10 cc, promptly after each of 4 periodic total-body doses of 168 r each at 8-day intervals, on experimental days 1, 9, 17, and 25. Physical factors were: 120 kvp, 9 ma, 0.25 mm Cu + 1.0 mm Al added filter, 30 cm mouse-target distance, 32 r/min. Experimental groups were similarly irradiated and treated in one of the following ways: (*a*) lead shielding of one thigh during each irradiation, as previously described (*2*); (*b*) intravenous injection of bone marrow cells suspended in Locke's solution; (*c*) intravenous injection of thymic cells similarly suspended; and (*d*) intraperitoneal implantation of minced skeletal muscle. The bone marrow suspensions were freshly made each time according to the method of Lorenz *et al.* (*4*). In most instances, the suspension injected into a single recipient contained the femoral marrows of a single young C 57 black donor (1 : 1 concentration), but in one experiment the femoral marrows of each two donor mice were divided among 5 recipients (2 : 5 concentration).

One group of donor animals received 1–4 periodic x-ray exposures (168 r) at 8-day intervals with both thighs shielded by a lead strip 1 cm wide and 3 mm thick. The shielded femoral marrow was removed promptly thereafter, suspended, and injected intravenously (1 : 1) into a correspondingly irradiated recipient. Skeletal muscle from the shielded thighs of the same donors was minced, and small fragments were loaded into trocars for implantation. Thymic cell suspensions were made by mincing thymuses of untreated donors in Locke's solution; they were injected intravenously in 1 : 1 concentration.

At predetermined intervals from 1 to 26 days after the last irradiation (experimental days 26–51), corresponding groups were sacrificed. The thymus, spleen, and pooled superficial lymph nodes (2 axillary, 2 inguinal) were rapidly excised, dissected free of fat and connective tissue, weighed on a torsion balance, and preserved in Bouin's fluid for histologic study.

The mean thymic weights of all groups are summarized in Table 1, and those of the control, marrow-injected, and thigh-shielded groups are compared in Fig. 1. Thymic weight recovered rapidly in the thigh-shielded groups and again overshot normal levels by day 51. The thymuses of animals given marrow cell suspensions began to regenerate appreciably later (day 33), but their rate of recovery thereafter roughly paralleled that of the thigh-shielded groups. The irradiated controls receiving Locke's solution alone showed a slight abortive rise in thymic weight between days 29 and 33, after which there was a secondary fall to initial post-irradiation levels which persisted through day 51. The differences between the mean thymic weights of the marrow and placebo-injected groups are significant at the 0.05 level at day 33 in one experiment, and again at day 40, and become increasingly great thereafter.

Marrow cell suspensions in 2 : 5 concentration did not significantly affect thymic weights at days 33 and 45, nor were muscle implants or thymic cell suspensions effective at day 51. In additional studies, pre-irradiated (450 r) marrow-cell suspensions were ineffective at day 29, but normal marrow cell suspensions were equally so at this time. "Stimulated" marrow from the femurs of thigh-shielded, irradiated

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TABLE 1

THYMIC WEIGHT IN IRRADIATED C 57 BLACK MICE

Expt. day	Day 26	Day 29	Day 33		Day 40	Day 45	Day 51	
Days after last irradiation	1	4	8		15	20	26	
Expt. no.	IV	I	II	IV	IV	II	III	IV
No. mice/group	(7–8)	(9–10)	(4)	(6–8)	(7–8)	(5–6)	(7–8)	(6–8)
No x-ray; age controls	—	56.7 ± 2.5*	—	—	53.0 ± 4.3	—	44.8 ± 1.8	47.9 ± 3.2
X-ray + Locke's solution I.V.	15.7 ± 0.5	13.6 ± 1.1	29.6 ± 2.4	24.2 ± 1.4	22.2 ± 1.4	18.2 ± 0.9	20.3 ± 0.7	16.7 ± 0.8
X-ray + marrow I.V. 2:5	—	—	22.1 ± 0.9	—	—	20.7 ± 2.0	—	—
X-ray + marrow I.V. 1:1	17.4 ± 1.0	15.4 ± 0.8	29.9 ± 3.1	32.1 ± 2.9	31.6 ± 4.3	34.9 ± 3.8	46.3 ± 5.8†	39.6 ± 4.3
X-ray with thigh shielded	14.7 ± 0.7	—	—	42.3 ± 3.1	49.0 ± 3.8	—	66.7 ± 1.8	65.6 ± 3.6
X-ray + muscle implant I.P.	—	—	—	—	—	—	22.4 ± 5.0	—
X-ray + thymic cells I.V.	—	—	—	—	—	—	25.9 ± 4.1	—

* Mean and standard error by $\sqrt{\frac{\Sigma(\bar{x}-x)^2}{n(n-1)}}$.

† Marrow taken from shielded donor femurs immediately after 1–4 periodic irradiations.

donors was not significantly more effective at day 51 than normal marrow. Perhaps the interval between such irradiation and the time of sacrifice must be increased to permit the shielded marrow to express an enhanced proliferative or regenerative capacity. There was no correlation between body weight and thymic weight in any of the irradiated groups. Body weight in the irradiated groups was significantly lower than in the controls and was not affected in any systematic manner by any of the treatments employed (Table 2).

Lymph node weights were markedly reduced by irradiation in all groups, and recovered more slowly than those of the spleen and thymus. Mean lymph node weights of the thigh-shielded and marrow-injected groups were significantly greater than those of the placebo-treated groups at day 51, but not earlier. There was no consistent effect of treatment on spleen weight.

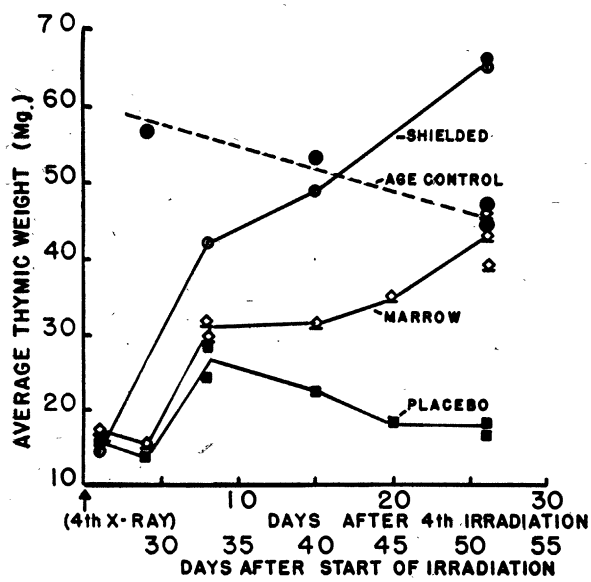

FIG. 1. Comparative effects of thigh shielding and intravenous bone marrow cell suspensions on thymic regeneration in C 57 black mice.

Histologic studies indicated an equal degree of initial radiation injury in thymus, nodes, and spleen of all groups. At day 40, there was much variation among the irradiated-placebo thymuses, some of which still showed marked cellular depletion and poorly defined cortical-medullary boundaries. Those of the marrow-injected mice, though still somewhat smaller than normal, were more uniformly restored to virtually normal architecture, and the thymuses of the thigh-

TABLE 2

Expt day	Expt no.	Treatment	Mean body wt ♂	Mean body wt ♀	Mean thymic wt
40	IV	Controls, no x-ray	23.4	19.4	53.0
		X-ray + Locke's solution	21.5	18.7	22.2
		X-ray + marrow I.V.	19.8	18.4	31.6
		X-ray + thigh shielding	20.3	17.6	49.0
51	III	X-ray only	22.8	19.1	23.2
		X-ray + Locke's solution	22.2	18.0	20.3
		X-ray + marrow I.V.	21.6	19.2	46.3
		X-ray + muscle I.P.	21.1	18.5	22.4
		X-ray + thymic cells I.V.	22.1	18.8	25.9
		Controls, no x-ray	24.7	23.1	44.8
		X-ray + thigh shielding	22.0	17.5	66.6
51	IV	Controls, no x-ray	23.8	20.0	47.9
		X-ray + Locke's solution	22.2	17.5	16.7
		X-ray + marrow I.V.	22.8	18.8	39.6
		X-ray + thigh shielding	22.0	18.3	65.6

shielded animals were larger and still more advanced in this respect. There appeared to be a greater degree of extramedullary hematopoiesis at days 40 and 51 in the red pulp of the irradiated-placebo spleens than in those of the other two groups. However, regeneration of the Malpighian corpuscles of the thigh-shielded and marrow-injected groups was evident distinctly earlier than in the irradiated-placebo controls.

It appears that intact bone marrow cells, whether autologously present in the shielded femurs of irradiated mice or injected into unshielded animals from homologous donors, may in some way facilitate regeneration of radiation-damaged lymphoid tissues

and, in particular, of the thymus. This phenomenon may explain in part the protective effect of homologous marrow cell suspensions and activated ectopic marrow against lethal doses of irradiation in mice and rats (*4, 5*). In view of the inhibitory effect of thigh shielding on the development of radiation-induced lymphoid tumors, it seems reasonable to predict that a similar, though perhaps less pronounced, inhibition of this neoplastic process will be observed after injection of exogenous marrow cells into irradiated mice.

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The Interaction of Genetic and Environmental Influences Affecting the Incidence of Avian Leucosis

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There is some doubt whether the various forms of avian leucosis, such as neural, visceral, and ocular lymphomatosis, erythroblastosis and granuloblastosis (the last two are uncommon), can be induced by a single virus, or whether several different viruses are involved, but there is agreement among pathologists and poultrymen that the disease is the most serious one afflicting domestic fowls today.

Most mortality from leucosis occurs between 2 and 16 months after hatching. In that period deaths from this disease may reach 50% or more. Recent tests of about 3300 severely exposed female chicks representing 36 different, improved strains showed that about 20% died of neoplasms (nearly all leucosis) before reaching 500 days of age (*1*).

In the light of present knowledge, the most important factors determining the mortality from leucosis in any exposed flock seem to be (*a*) the genetic constitution of the birds, (*b*) the age at which they are exposed, and (*c*) the severity of that exposure. This last apparently depends in part upon environmental conditions still unknown. It is the purpose of this note to show how these 3 factors interact and how knowledge of that interaction can be used to attain a satisfactory measure of control.

(*a*) Genetic resistance to leucosis has been studied continuously by the writers for the past 18 years. The feasibility has been demonstrated of breeding strains of White Leghorns capable of high egg production and so resistant that, when severely exposed to leucosis, mortality from that disease is almost negligible. Previous reports (*2, 3*) showed that deaths from neoplasms among these birds up to 500 days of age in 3 successive years, 1944–46, varied only from 5–8% in unculled flocks. In the birds hatched in 1951, mortality from leucosis alone (excluding other neoplasms) in that same period among 2177 females of the C- and K-resistant lines was only 2.7%, but it was 61% for birds of the susceptible strain. These had been mixed with the others since hatching and even during that process.

(*b*) The relation of age to susceptibility became clear when different investigators showed independently that chicks not genetically resistant to leucosis, when reared in complete isolation until about 5 months of age, are then highly resistant when brought to infected premises (*4, 5*). Subsequent experiments in which chickens reared in isolation, and hence presumably uninfected, were taken at various ages to infected quarters showed that most of this resistance had been developed by 10–16 weeks (*6, 7*) and in some cases even at 6 weeks (*8*) or 30 days (*9*). The utilization of this knowledge for control of the disease has been difficult, and in most cases impracticable, because few poultrymen can afford to maintain a separate, isolated establishment for rearing their birds.

(*c*) The severity of natural exposure varies greatly from flock to flock within any one area, from year to year in any one flock, and even from one weekly hatch to the next at any one farm. Among environmental factors that might contribute to this variability, only one has been clearly incriminated, namely, the proximity of the chicks to adult fowls during the critical first few weeks after hatching. During 3 successive years, deaths from neoplasms (mostly lymphomatosis) up to 500 days of age among birds that had been brooded only 40 feet from adult stock were 1.6–4 times as numerous as in full sisters that had been brooded over 100 feet from older birds (*10*). Although that difference in environment, with its accompanying difference in severity of exposure, was maintained only during the critical first 13 days from hatching, after which all chicks were kept on the same rearing range until 5 months old, it proved to be the deciding factor (apart from genetic resistance) in determining the amount of mortality from leucosis in these birds at later ages.

The relation of these three interacting factors—inheritance, age at exposure, and severity of exposure—to mortality from leucosis is clearly revealed in Fig. 1, which shows the mortality from that disease in the 4 annual generations of our White Leghorns raised in the years 1948–51. All females alive at 6 weeks of age are included, and, for simplicity, those of the 2 resistant strains are shown as a single group.

In 1948, 1949, and 1951, chicks exposed right after hatching apparently underwent the usual severe exposure desired to facilitate selection, and, as a result, mortality from leucosis in the susceptible strain ranged from 51–64.5%. Among birds of the resistant strains, which had been brooded, raised, and maintained with the others, corresponding mortality was only 2.7–7.8%.