Relative Effect of Thymocytes from Irradiated Donors on Hemopoiesis in P → F_1 Chimeras[1] (36475)

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Interest in the functional role of the thymus in hemopoiesis has increased considerably (1–5) since it was demonstrated that thymocytes enhance all elements of spleen growth *in vitro* and *in vivo* (6, 7). Goodman *et al.* (1, 2) have shown that the degree to which thymocytes augment hemopoiesis in parental ($P \rightarrow F_1$) chimeras (heavily irradiated B6D2F_1 recipients of B6 bone marrow) is dependent on age of thymus donors. In addition, there is an inverse relationship between donor age and cell yield insofar as augmentation by thymocytes is concerned. Although a preliminary histologic examination (3) revealed no obvious differences in the cortex-to-medulla ratio, which confirms Metcalf's (8) finding, it was reasoned that the age-dependent changes in augmentative capacity reflected modifications in concentration of the effective cell type in the thymus. Presently, however, little is known about the specific function of the cell types found in the thymus, making it difficult to relate an observed functional effect to a specific cell type.

Trowell (9, 10) has reported that small lymphocytes in the cortex of the rat thymus are four times more radiosensitive than those of the medulla, and similar results have been reported for the mouse (11). On the assumption that the reticular cell type is unchanged, Trowell also showed that thymocytes remaining in the cortex and medulla *in vivo* are dependent on the dose of X-radiation He found that 2 days after 200 R of whole-body X-irradiation the thymocytes remaining in the cortex and medulla were 1.9 and 31.9%, respectively, of the original population. Thus, exposure to 200 R provides a differential effect on the cell types of the thymus in the following decreasing order of damage: cortical thymocytes, medullary thymocytes, reticular cells. These results, and those previously obtained in this laboratory (3), suggested alteration of the normal ratio of cell types by radiation as an approach to determine which type of thymocyte augments hemopoiesis.

The experiments presented here were designed to determine the effectiveness of thymocytes from irradiated and normal donors in augmenting hemopoiesis in P → F_1 chimeras.

Methods. Thymus and marrow donors were C57 Bl/6 Jax mice (or B6) 8–12 weeks old. Recipients were 12–15-week-old (B6 ♀ × DBA/2)F_1 (or B6D2F_1) mice. Both males and females were used, and donors and recipients were matched for sex.

Donors and recipients were exposed to X-rays from a unit operated at 250 kVp under the following conditions: 145 R/min, 15 mA, 1 mm Al added filtration: hvl = 0.5 mm Cu, target–object distance 60 cm. Donors were exposed to 200 R and recipients to 900 R of whole-body X-radiation, the latter a dose slightly more than the 30-day LD_{99}.

Recipient mice were given 5×10^5 B6 marrow cells and varying doses of thymocytes intravenously 18–24 hr after irradiation. Because of the difficulty sometimes encountered in giving large doses of thymocytes iv, it was necessary to inject these cells, usually in a volume of 0.5 ml, slowly. As an

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TABLE I. Splenic Colonies in Irradiated (900 R) B6D2F$_1$ Mice Given 5×10^5 B6 Bone Marrow Cells and Varying Doses of B6 Thymocytes.

Experiment[a]	Thymocyte dose (millions)	Thymocyte donor	
		Normal	Irradiated[b]
		(mean no. colonies ± SE)[c,d]	
A(♂)	0	9.7 ± 1.1 (14)	
	4	15.5 ± 0.9 (20)	27.9 ± 1.4 (19)
	8	24.5 ± 1.4 (17)	31.5 ± 1.5 (19)
	16	24.9 ± 0.6 (19)	41.8 ± 1.6 (18)
B(♂)	0	9.1 ± 1.1 (15)	
	4	11.3 ± 1.5 (19)	17.1 ± 1.7 (15)
	8	22.3 ± 1.2 (20)	23.2 ± 1.2 (15)
	16	25.6 ± 2.7 (19)	32.4 ± 0.6 (16)
C(♀)	0	3.7 ± 0.7 (21)	
	4	6.9 ± 1.0 (28)	12.8 ± 1.0 (29)
	8	13.4 ± 1.3 (27)	22.2 ± 1.1 (30)
	16	17.0 ± 1.0 (29)	31.6 ± 0.8 (25)

[a] Symbols in parentheses indicate sex of donor and recipient mice.

[b] Exposed to 200 R of whole-body X-radiation 72 hr before thymocytes taken.

[c] Number of recipient mice in parentheses.

[d] All values are significantly different at the $p < 0.01$ level, except in Expt. B at the 8-million thymocyte dose, where the p value is < 0.05.

added precaution against death from embolism, mice of all groups were given an ip injection of 50 units of heparin in 0.5 ml saline 10–20 min before cells were to be given iv. Marrow cells were injected in a volume of 1 ml phosphate-buffered saline (PBS) 1–4 hr after thymocytes. Details of this procedure have been described (1). Animals were housed five per cage after treatment and had free access to food (Purina Chow) and water. Daily mortality checks were made on treated mice.

The spleen colony techniques of Till and McCulloch (12) was used to measure colony-forming units (CFU) from the transplanted marrow. Eight days after injection of cells, spleens from recipients were removed and fixed in Tellyesniczky's fluid. The following day a dissecting microscope (15×) was used to count surface colonies. Differences were analyzed by the Student's *t* test.

For histologic study thymus tissues from normal or irradiated donors were fixed in Zenker-formal, sectioned, and stained with hematoxylin and eosin. Feulgen fast green (associated with DNA) and methyl green pyronine (associated with RNA) were used in tests for specific histochemical properties.

Results. At every cell dose thymocytes from irradiated donors were significantly more effective than those of normal mice in enhancing spleen colony formation (Table I). The difference was almost twofold in two cases (experiments A and C) and was smaller in the third (experiment B). The control level (*i.e.*, the number of splenic colonies produced by 5×10^5 marrow cells alone) for females (experiment C) was around half that for males (A and B), a finding that confirms data of McCulloch and Till (13), who have shown that for 10^5 parental marrow cells female mice develop about 30% fewer colonies than do males. If we can assume a linear relationship between CFU and marrow dose, our value for males is 1.9 colonies (0.7 for females) per 10^5 marrow cells, compared to McCulloch and Till's finding in a different $P \rightarrow F_1$ combination of 1.1 colonies (0.4 for females).

In contrast to the ^{59}Fe-uptake method (5), the assay for colony formation in our experiments (when thymocytes were taken from ir-

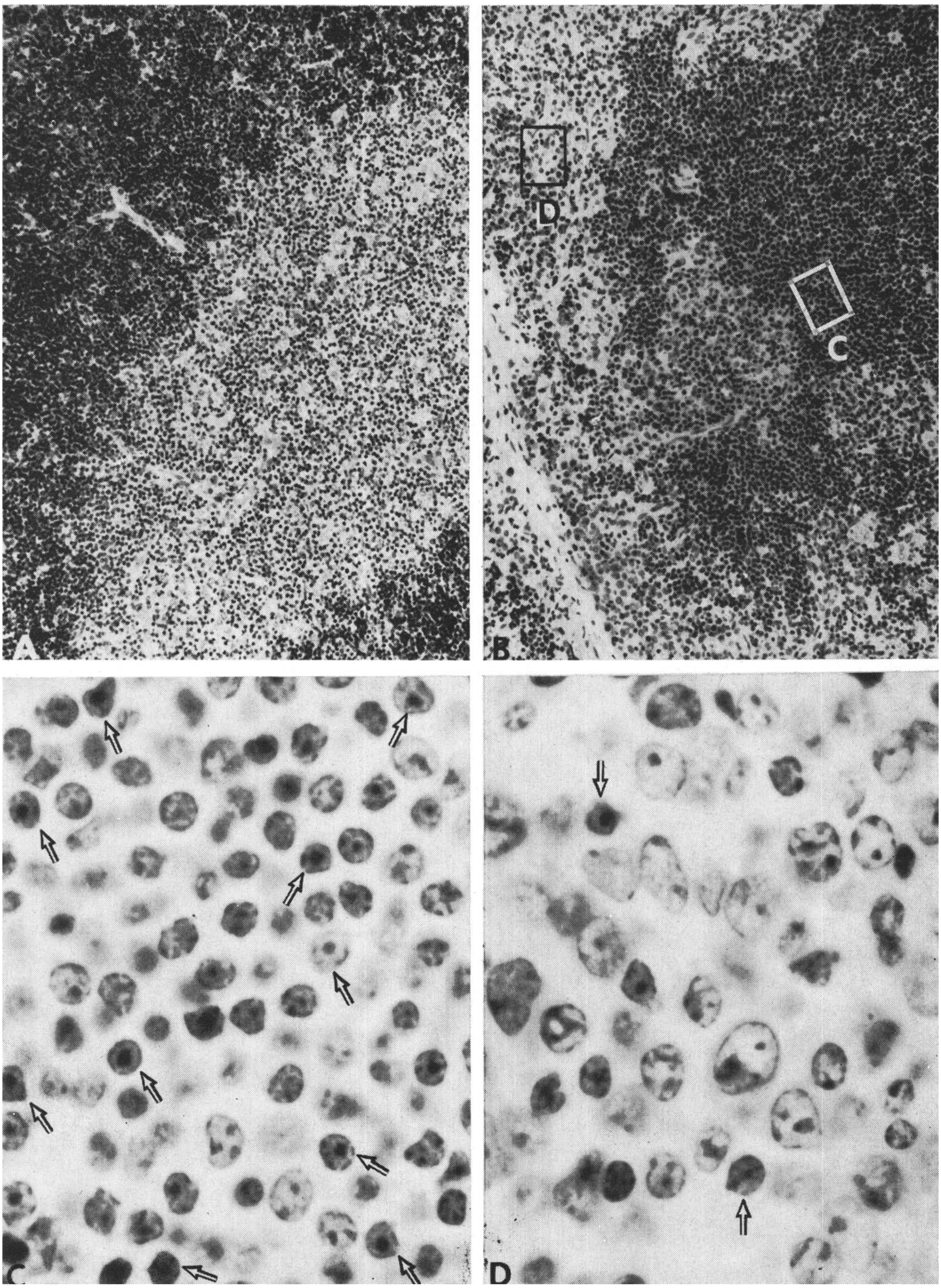

FIG. 1. Differential histology of thymus sections (medullary and cortical zones) from irradiated and unirradiated young-adult male mice. A. Normal; hematoxylin–eosin (H–E) 80×. B. 72 hr after 200 R; H–E, 80×. Note thymus inversion (*i.e.*, increase in number of thymocytes in medullary area). Areas within rectangles are approximately the same areas depicted in the enlargements (below). C. 1280× view of medullary zone from irradiated thymus; H–E. Note that many cells have dense nucleoli (arrows). D. 1280× view of cortical zone from irradiated thymus; H–E. Cells containing dense nucleoli are considerably less frequent than in Fig. 1C.

radiated donors) shows at least a twofold augmentation of hemopoiesis when the ratio of thymocytes to marrow cells is 8:1. This is a much smaller value than the lowest ratio with normal thymocytes (40:1) at which augmentation was measured by the ^{59}Fe-uptake method. Results of the colony-forming assay confirm previous work (3) showing that the augmentation is not restricted to erythropoiesis but includes all proliferative elements of bone marrow.

The effect of 200 R on the histology of the thymus is shown in Fig. 1. Atrophy and reduced cell density caused by the radiation are clearly present. At the lowest magnification (Fig. 1B), the medulla appears darker and denser than the cortex (*cf.* Fig. 1A), an observation which has been described and called thymus "inversion" (9, 14). Notably absent in the irradiated thymus at 72 hr are the cellular debris and phagocytosis characteristic of the early destruction of lymphocytes (see Figs. 1C and 1D, also Refs. 9–11).

Cortical and medullary zones of irradiated thymus contain a few lymphocytic cells, which appear in greater concentration in the medulla (Fig. 1C) than in the cortex (Fig. 1D). In both zones these lymphocyte-like cells have condensed and dark nucleoli, similar to those reported by Potmesil *et al.* (11) in radiation studies on mice. Especially in the cortex these same cells, probably reticuloepithelial in origin, give a markedly positive reaction to methyl green pyronine, but almost no reaction to Feulgen fast green. Ring-shaped nucleoli are seldom present in such cells.

Discussion. The present results confirm and extend the data of Goodman *et al.* (3), who suggested that enhancement of bone marrow growth by thymocytes might involve a cell-to-cell interaction between elements derived from both bone marrow and thymus. It is not yet known, however, which of the cell types normally found in the thymus is involved. To our knowledge no evidence has been reported that humoral factors are involved in the enhancement of hemopoiesis by thymocytes.

Our results show that irradiation of the thymus increases the enhancing effect of thymocytes on bone marrow growth and reduces the ratio (thymocytes to marrow cells) at which this effect can be detected. The basis for greater enhancement by irradiated thymocytes may involve differential cellular radiosensitivity, since we are concentrating a particular cell type (or types) in the 200-R donors.

There appear to be two distinct cell populations in the thymus: epithelial–reticulum cells and lymphoid cells. The former are more radioresistant than the latter according to Trowell *et al.* (9) and Potmesil *et al.* (10). The lymphoid population appears to be represented (10) by at least two subcompartments, one sensitive and the other less sensitive. The less sensitive compartment can be characterized morphologically as large, immature blast cells having a D_{37} of 400 to 500 R, whereas the sensitive subcompartment is represented by more mature cell types with a D_{37} of about 140 R (11). If these values are correct, the relative number of less sensitive lymphoid-like cells in our 200-R donors should have increased.

We have also noted (Salinas, Smith, and Goodman, unpublished results) that the augmentation of poor growth by irradiated thymocytes appears to be associated with an increase in mean-cell volume of the thymocyte population. We suggest that this less sensitive, large blastic cell type (which, by its staining properties, shows increased RNA synthesis) is the one involved in enhancement of marrow growth.

Summary. Thymocytes exposed *in vivo* to 200 R of X-rays 72 hr prior to transplantation enhanced marrow growth almost twice that seen in mice similarly treated with nonirradiated thymocytes. Growth of marrow was evaluated by the spleen colony-forming technique. These results confirm and extend previous data from this laboratory for which a cell-to-cell interaction has been postulated as a basis for augmentation of bone marrow growth by thymocytes. The present data show a minimal effective ratio (thymocyte: marrow cell) of 8:1 for irradiated thymocytes compared to a value of 16:1 for un-

irradiated thymocytes.

On the basis of our results and the known differential radiosensitivity of thymocytes, we suggest that the less radiosensitive blastic lymphoid cells of the thymus are responsible for augmentation of poor growth of parental bone marrow transplanted into lethally X-irradiated F_1 mice.

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