Radiation-Resistant Thymic Stem Cells[1] (37065)

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While the evidence for radiation sensitivity of hematological stems cells of the adult is overwhelming, less is known about the sensitivity of embryonic stem cells. Moreover, the radiation sensitivity of subpopulations of cells responsible for differentiation into erythroid, myeloid, or lymphoid cells of the developing organs of the embryo may not necessarily be the same. Our own interest in the development of the embryonic thymus rudiment of the mouse prompted an investigation into the effects of irradiation on the cells responsible for differentiation into embryonic thymic lymphocytes.

Materials and Methods. Thymus rudiments were obtained from mouse embryos of 12–13 days of embryonic age as determined by vaginal plug timed matings and precise staging on the basis of submandibular gland morphology; the latter provides a more accurate determination of developmental equivalence. The stage employed in these studies may be considered equivalent to Moore and Owen day 12.5 animals (1). Salivary gland mesenchyme was obtained by removal of the apical mesenchymal cap of dissected submandibular gland rudiments; trypsin was not used [cf. (2, 3)].

For *in vitro* studies thymus rudiments were placed in the well of a Millipore filter assembly (4) to permit lymphoid differentiation to occur. Tissue combinations were made simply by placing appropriate tissues in immediate contact in the filter well to permit fusion. The medium consisted of Eagle's basel medium supplemented with 10% horse serum (Gibco), 5% chick embryo extract (9-day embryos, 50% in Tyrode's solution) and antibiotics. Medium was changed at about 2-day intervals.

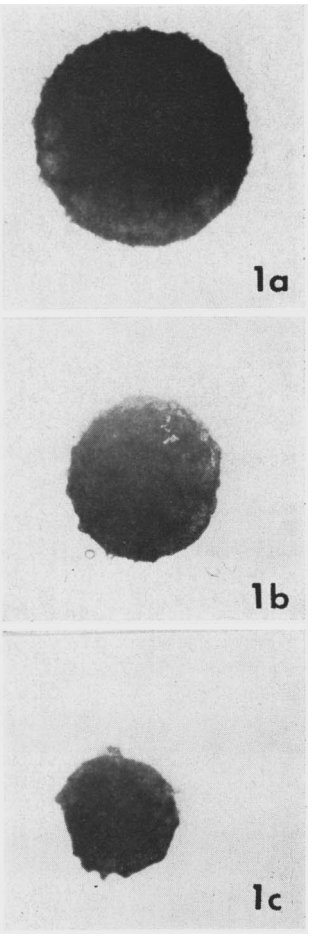

FIG. 1. 13-day thymus rudiments after 7 days *in vitro* (R): (a) 0; (b) 500; (c) 1000. [40×].

Irradiation was performed on a GE Maxitron 300 kVp X-ray machine, with dose monitored by a Victoreen dosimeter, and administered at a dose rate of approximately 120 R/min with intervening 1 mm Al and 0.5 mm

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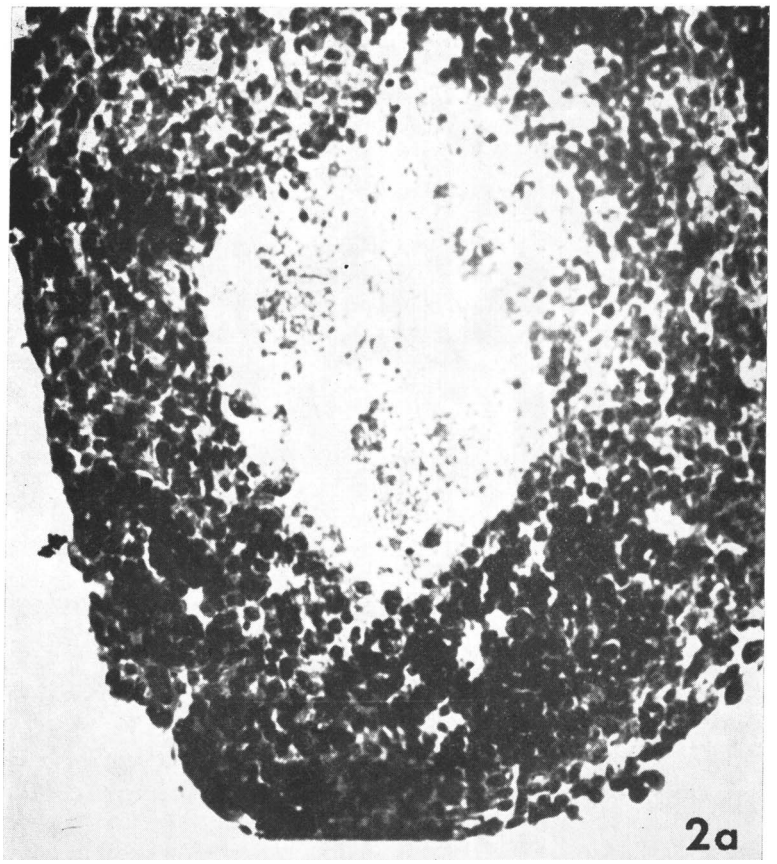

FIG. 2a. Histological preparation of 7-day cultures of 13-day thymus rudiments (R):0 R.

Cu filtration. Adult animals were irradiated on a rotating turntable. Irradiation of cultures was carried out with tissue placed in freshly dispensed horse serum–Tyrode's (1:1) solution.

Eye grafts were made in standard fashion [cf. (2)] with Avertin used as anesthesia. Histological examination was carried out on grafts fixed with Bouin's solution and on cultures fixed in Zenker's. Sections were routinely stained with hematoxylin and eosin.

Cell number and volume of thymic lymphocytes were determined on representative cultures by analysis on a Nuclear-Chicago multichannel analyzer employing the Coulter principle for generation of impulses.

Results. Thymus rudiments were irradiated with 0, 250, 500 or 1000 R of X-rays and then grown *in vitro* for 7 days; a total of 80 rudiments was analyzed. Irradiated thymuses showed the effects of exposure by decreased growth; 1000 R was sufficient to prevent any overt increase in explant size during the culture period (Fig. 1). When representative cultures were examined histologically, however, it was observed that lymphoid differentiation had occurred in all experimental groups. Explants irradiated with 1000 R, even though small, were comprised largely of lymphoid cells, with the effect of irradiation being manifested primarily by the reduction in size and restriction in follicular organization (Fig. 2). The distinct impression gained from histological preparations was that irradiation affected primarily the expansion of thymus connective tissue elements.

Irradiated and control thymus rudiments were also studied by transplantation into the anterior eye chamber of adult lethally irradiated animals; a total of 15 transplants was studied. Previous work from this laboratory (12) had established that under these

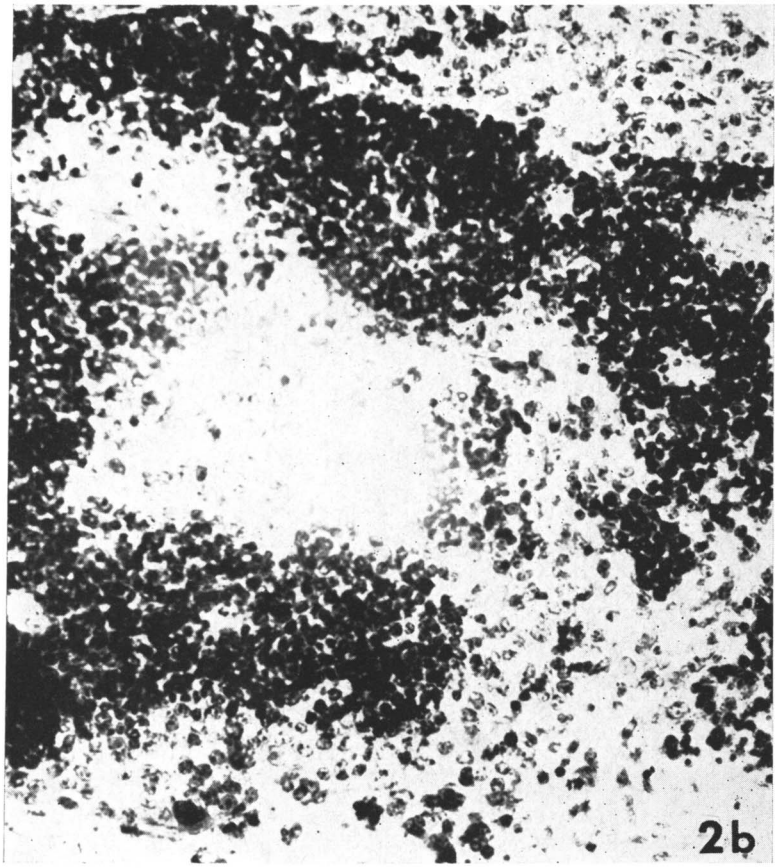

FIG. 2b. Histological preparation of 7-day cultures of 13-day thymus rudiments (R):1000 R.

conditions, host cell migration into grafted rudiments does not occur during the 7-day experimental period. The results of our studies were essentially the same as those observed in the *in vitro* experiments. Thus, while there was a marked effect on growth, implants uniformly showed a predominant lymphoid cell population, independent of the dose of irradiation received (Fig. 3).

Since thymus cell populations have a characteristic size distribution pattern [cf. (5, 6)] pooled thymus cultures (20 rudiments/assay) were prepared as cell suspensions and compared by cell size analysis on a multichannel size analyzer. As shown in Fig. 4, the size distribution pattern of thymic lymphocytes was essentially the same for both groups, with both irradiated and unirradiated cultures showing a characteristic peak at *ca.* 140 μm^3, typical of the newborn thymic lymphocyte.

To examine the possibility that irradiation effects were exerted primarily on the connective tissue elements rather than on prelymphoid stem cells, irradiated thymic rudiments were provided with unirradiated salivary gland mesenchyme (2) and cultured for 7 days; a total of 26 cultures was studied. Here the results were variable. In the best cases salivary gland mesenchyme caused extensive growth and enhanced development of follicular structures; in other instances mesenchyme seemed quite ineffective. The total lymphoid cell numbers at any rate, did not appear to be affected by the presence of unirradiated mesenchyme.

Discussion. At the present time it is generally accepted that the development of the thymus involves initially a migration of yolk-sac derived stem cells which enter the early rudiment of the thymus (1, 7–9), after which time the self-differentiation of that rudiment into a lymphoid organ can occur both *in vitro*

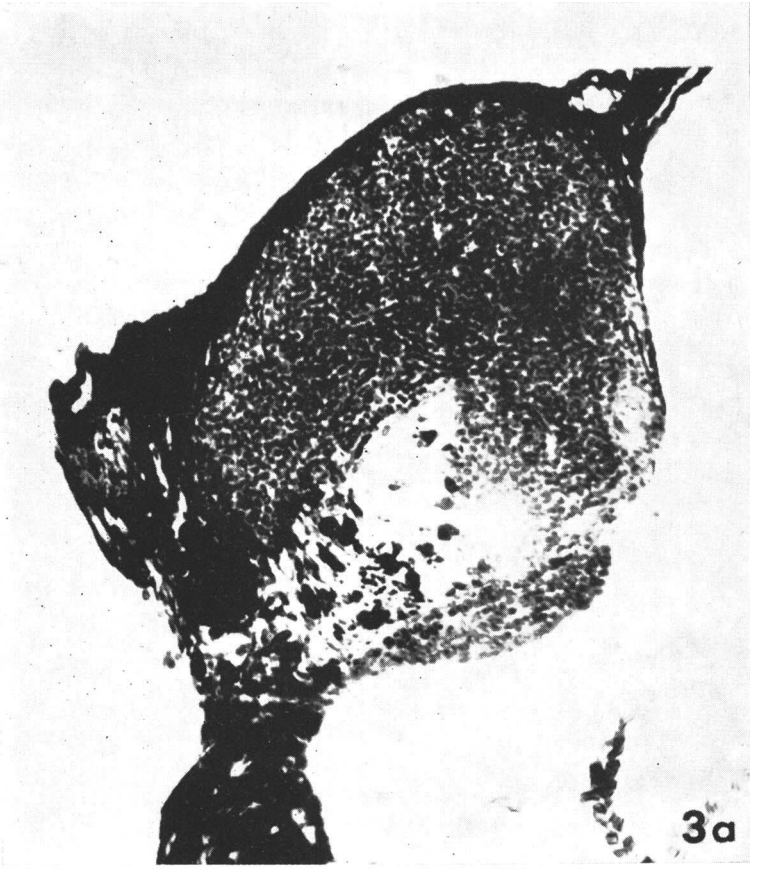

FIG. 3a. Histological preparation of 13-day thymus rudiment irradiated with 1000 R and grown in the anterior eye chamber of a syngeneic lethally irradiated adult mouse for 7 days.

(4, 3) or after transplantation into irradiated host animals (2, 10–13). Earlier studies had suggested that thymus epithelial cells convert into lymphocytes (3); more recent evidence of the yolk-sac origin of stem cells of the thymus (cited above) is convincing, but does not exclude the possibility that subpopulations of thymic lymphocytes may include cells of differing embryonic origins (14, workshop No. 9; 15). No matter what the initial source of lymphoid cells is, the thymic microenvironment clearly plays an essential role in permitting differentiation of cells to become mature thymocytes (2, 8, 16).

Why the thymus lymphoid differentiation that we have studied should be so refractory to irradiation is not altogether clear. In general, lymphoid cells are radiation sensitive and even the more resistant large lymphocyte [(17, 18), cf. (19)] is sufficiently radiation sensitive to preclude its implication in our observed results. It seems reasonable to suggest that the stem cell, after migration into the rudiment of the thymus, is quiescent and radiation resistant until it is stimulated to proliferation, an event which occurs in the mouse at about 14 days of development; hence the stage used in our studies represents that null period in thymic development after stem cell immigration and prior to stem cell proliferation under the inductive stimulation of the thymic microenvironment.

That so many lymphoid cells do arise even after 1000 R treatment does lend some support to the notion that the preponderant cell population of the 12–13 day thymus anlage is competent to differentiate into lymphocytes. If this is indeed so, then the prelymphoid cell cannot be restricted to the Giemsa-positive, migratory stem cell population reported by Moore and Owen (1) and identified ultrastructurally by Mandel

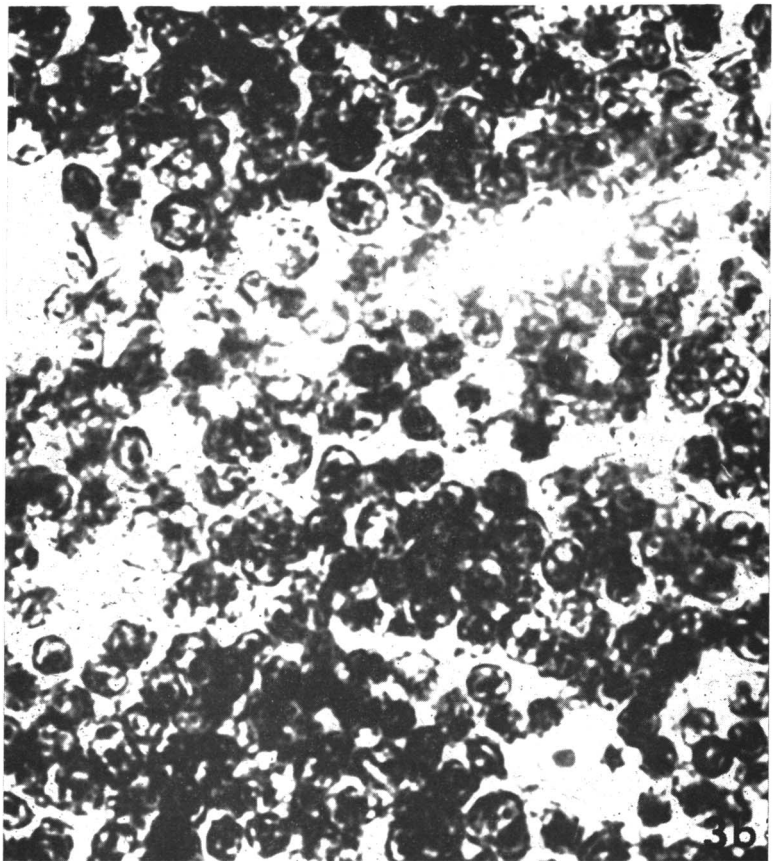

FIG. 3b. Histological preparation of 13-day thymus rudiment irradiated with 1000 R and grown in the anterior eye chamber of a syngeneic lethally irradiated adult mouse for 7 days.

and Russell (16). Whatever the explanation for the resistance of thymic lymphocyte precursor cells to irradiation, however, several points should be emphasized:

1. If the cell line derived from radiation-resistant embryonic thymic cells is distinct from other thymic lymphocytes derived from recirculation or from bone marrow, this line, a minority population, may have unique immunological function.

2. Although studies of radiation chimeras have emphasized the role of donor cells in recovery, a host cell recovery is not precluded even after "lethal" irradiation, and the absence of demonstrable host cells may reflect donor cell surveillance, lack of sensitivity of assay procedures, or the absence of a physiological impetus to development.

3. The ultimate fate of transplanted tissues, even after treatment of the host with radiation, cytotoxic and immunosuppressive agents may depend on a minority component of the thymus which is essentially embryonic in nature and which is refractory to destruction by the usual agents employed for lymphoid cell elimination.

Summary. 1. Thymus rudiments obtained from 12 to 13 day mouse embryos were irradiated with 250, 500 or 1000 R X-rays and their subsequent morphogenesis studied both *in vitro* and by transplantation into irradiated host animals.

2. Although irradiation had a marked effect on total growth of rudiments, the differentiation of lymphoid cells did not appear to be inhibited.

3. Both on the basis of morphology in histological preparations and by size analysis, lymphocytes formed after irradiation were indistinguishable from lymphocytes produced by unirradiated rudiments.

4. The results suggest that a significant

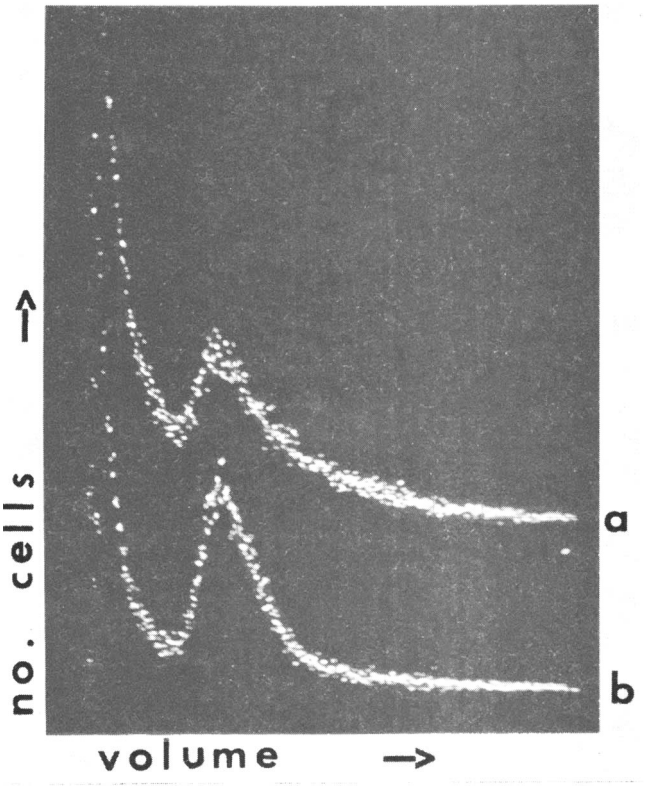

FIG. 4. Size distribution pattern of cell suspensions obtained from 13-day thymus rudiments after 7 days *in vitro* (R): (a) 0; (b) 1000.

portion of lymphoid precursor cells of the early embryonic rudiment of the thymus are radiation resistant.

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