HEMATOPOIETIC THYMOCYTE PRECURSORS*

I. Assay and Kinetics of the Appearance of Progeny.

BY JULIAN L. KADISH‡ AND ROSS S. BASCH§

(From the Department of Pathology, New York University School of Medicine, New York 10016)

The thymus is an encapsulated compound organ consisting of lymphocytes interlaced with a supporting network of thymic epithelium (1). These lymphocytes are the source of peripheral "T" or thymus-derived lymphocytes. Experiments using either chromosomally marked parabiotic pairs (2) or the transfer of chromosomally or antigenically identifiable hematopoietic cells into lethally irradiated hosts (3–7) have indicated that in adult animals the thymic lymphocyte does not arise from endogenous thymic elements. Results in both adult and embryonic animals indicate that thymocytes are derived from hematopoietic precursor cells (8–9). These peripatetic progenitor cells enter the thymus via the blood stream and differentiate therein. Entry of the injected cells has not been observed directly, and thymocytes of donor origin are generally not found in thymus until 2 wk after injection (3, 5, 6). Because of this, characterization and quantification of the hematopoietic thymocyte precursor has proved difficult. Proliferation of the progeny of an intrathymic radioresistant thymocyte precursor immediately after irradiation has further obscured the study of hematopoietic thymocyte precursors (10).

Thus, despite the fact that the stem cell-end organ relationship of the thymus and the hematopoietic tissues has been known for more than a decade, neither the precise nature of the thymic influence nor the identity of the prethymic stem cell has yet been established. This report describes a quantitative assay for this stem cell. The assay makes use of the availability of mice bearing allelic Thy 1 (θ) genes to provide a marker to distinguish between donor and host cells. Thus AKR inbred mice obtained from Cumberland View Farms, Clinton, Tenn. (AKR/C) possess the Thy 1.2 antigen (θ-C_3H) instead of the expected Thy 1.1 antigen (θ-AKR) generally found in AKR mice. The difference in Thy 1 antigen type is the only alloantigenic difference between these mice that has so far been identified (11). We have made use of this difference to identify the progeny of hematopoietic cells from an AKR/T mouse, proliferating in an irradiated AKR/C recipient, and this has enabled us to examine the dynamics of the repopulation of the thymus in the irradiated mouse.

Materials and Methods

Mice: Source and Care. Female mice, 5–6-wk old, were obtained from The Jackson Labora-

* This work has been supported by U. S. Public Health Service Grant AI 1132 and contract NCI CE 43927.

‡ Medical Scientist Trainee (Grant 2T 05 GM 0668).

§ Recipient of Career Development Award HD 34968.

tory, Bar Harbor, Maine (AKR/J), Texas Inbred, Houston, Tex. (AKR/Texas, hereafter designated as AKR/T), and Cumberland View Farms, Clinton, Tenn. (AKR/Cumberland, hereafter designated as AKR/C). They were housed 3–4 per cage, fed commercial mouse food and oats, and given acidified, chlorinated water (pH 3; free chlorine, 12–20 ppm).

Antisera and Complement. Anti-Thy 1 antisera (anti-θ) were produced utilizing the Thy 1 difference between AKR/C and AKR/T mice. Mice from each strain were immunized by intraperitoneal injection of thymocytes from the other strain. The initial dose was followed 1 mo later with two biweekly injections, and the mice were bled 10 days after the second injection. One thymus was used to immunize three mice. In general, this procedure yielded anti-Thy 1.2 with a titer of 1:400 vs. AKR/C thymocytes and anti-Thy 1.1 with a titer of 1:3,000 vs. AKR/T thymocytes. When necessary, these sera were absorbed with syngeneic thymus and spleen cells to remove autoantibodies.

Rabbit serum selected for low toxicity to thymus cells was prepared for use as a source of complement. The serum was stored at −70°C in 0.5-ml aliquots until used. The complement was generally used at 1:25–1:30 final dilution in tissue culture medium RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.).

Cell Suspension Preparation. Cells were prepared in tissue culture medium RPMI 1640 containing 50 U/ml penicillin and 50 μg/ml streptomycin and supplemented with bovine serum albumin (BSA,[1] 7 mg/ml; 2 ml of Pathocyte 5, Miles Laboratories, Inc., Elkhart, Ind. per 100 ml of medium). Bone marrow suspensions were prepared from femurs by cutting off both ends and blowing out the marrow plug with medium using a needle and syringe. The marrow was resuspended with a pipette, and the debris was allowed to settle. The cells were washed once by centrifugation before use. Suspensions of spleen, lymph node, and thymus cells were prepared by teasing the organ apart with a scalpel and needle and gently pressing the pieces through a stainless steel screen (200 μm mesh). After centrifugation and resuspension, the cells were filtered through two layers of filtering silk to remove macrophage aggregations.

Cell Counts. All cell counts were made on a Coulter Model A Electronic cell counter (Coulter Electronics Inc., Hialeah, Fla.). Where appropriate, Zap-Isoton (Coulter Electronics Inc.) was used to lyse red cells before counting.

Assay for Donor Cells: Inhibition of [^{3}H]TdR Incorporation by Specific Antisera. Donor cells were identified in a cytotoxic test exploiting allelic differences in Thy 1 (θ) surface antigen present on the thymocytes of AKR mice purchased from different sources. AKR/T mice bear the Thy 1.1 (θ-AKR) alloantigen, while AKR/C mice bear Thy 1.2 (θ-C3H). Cells from donors bearing the Thy 1.1 allele were injected into irradiated animals bearing the Thy 1.2 allele. At various times the proportion of replicating thymocytes derived from donor hematopoietic cells were determined by selectively killing thymocytes bearing one of the Thy 1 (θ) alleles and comparing the incorporation of tritiated thymidine ([^{3}H]TdR) by the surviving cells with that of the whole population.

Thymocytes to be tested were prepared as a single cell suspension at a concentration of 2×10^6 cells/ml in RPMI 1640 plus antibiotics and 0.7% BSA. 100 μl were pipetted into the wells of a flat bottom microtiter plate (Microtest II, Falcon Plastics, Oxnard, Calif.). Some wells received no antiserum, other received antiserum against host thymocytes, and a final control group received antisera against both Thy 1 types. It was thus possible to assess differentially the total [^{3}H]TdR incorporation and the proportion due to the proliferation of the engrafted donor cells. The contents of the wells were mixed on a microplate mixer (Micro Mixer, Cooke Laboratory Products, Alexandria, Va.), and the plate was incubated at 4°C for 30 min. After centrifugation at 1,000 rpm for 10 min, the supernate was carefully aspirated and 100 μl of 3% fresh rabbit serum (diluted in RPMI 1640 plus antibiotics and BSA) was added to each well as a source of complement. After a 20 min incubation at 37°C in an atmosphere of 5% CO_2 in air, 0.5 μCi of [^{3}H]TdR (sp act 20 Ci/mM, New England Nuclear, Boston, Mass.) in 25 μl of RPMI 1640 was added to each well. After mixing, the plate was incubated at 37°C for 2 h. [^{3}H]TdR incorporation was measured as previously described (10) using an automated cell harvester. The radioactivity of the filters was counted in a toluene based acintillation mixture (Permafluor, Packard Instrument Co. Inc., Downers Grove, Ill.) in a Packard Model 3380 scintillation spectrometer. The proportion of dividing donor cells was

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; TCA, trichloroacetic acid.

calculated according to the following formula:

$$\text{proportion donor type thymocytes} = \frac{[^3\text{H}]\text{TdR incorporation in presence of antihost type Thy 1 antiserum} - [^3\text{H}]\text{TdR incorporation in presence of antisera to both Thy 1 alleles}}{[^3\text{H}]\text{TdR incorporation in absence of anti-Thy 1 antiserum} - [^3\text{H}]\text{TdR incorporation in presence of antiserum to both Thy 1 alleles}}$$

The percentage of donor type cells was calculated by multiplying the above by 100.

Alternatively a repopulation index (RI) could be calculated as follows:

$$\text{RI} = \frac{\text{proportion donor type cells}}{(1 - \text{proportion donor type cells})}.$$

This index offers the empirical advantage that it is a linear function of the number of injected cells. Data are presented in this form when the proportion of donor cells exceeds 20%. Below this the simpler percentage of donor type cells is used.

Distribution of Injected Cells. The distribution of injected nucleated bone marrow cells in irradiated recipients was determined after labeling the cells with ^{51}Cr by the method by Wigzell (12). After removing erythrocytes by incubating the cell suspension in isotonic Tris-buffered ammonium chloride (13), 10^7 cells were injected intravenously into groups of five animals. 24 h later the animals were killed and the amount of ^{51}Cr deposited in the various organs determined by counting the radioactivity within an organ in an automated gamma spectrometer (BioGamma, Beckman Instruments Inc., Fullerton, Calif.).

Irradiation. A ^{137}Cs source (Model M Gammator, Radiation Machinery Corporation, Parsippany, N. J.) delivering 630 rads/min was used to irradiate the mice used in these experiments. Recipients usually received 760 R whole body irradiation except where stated in the text.

Radioautography. Regenerating thymocytes were incubated with [^{3}H]TdR as described above. Aliquots were deposited on glass microscope slides using a cytocentrifuge (Shandon Southern Instruments Inc., Sewickley, Pa.). They were dipped in NTB-3 nuclear track emulsion (Eastman Kodak, Rochester, N. Y.) exposed for 2–3 wk, developed for 6 min in D-19 (Eastman Kodak Co.), fixed, and stained with hematoxylin. Fixed sections were prepared as previously described (10).

Assay for Proliferation of Hematopoietic Cells in Irradiated Mice. To determine the degree to which injected hematopoietic cells proliferate in the recipients, the rate of [^{3}H]TdR incorporation into trichloroacetic acid (TCA) insoluble material by the spleen and thymus, and the size of these organs were measured. Each irradiated mouse received 760 R and 20×10^6 spleen cells from either AKR/C or AKR/T mice. At appropriate times each mouse was injected intravenously with 25 μCi of [^{3}H]TdR (sp act 2.0 mCi/μM). 1 h later the spleens and thymuses were removed, weighed, and homogenized. Macromolecular material was precipitated with 10% TCA. The precipitate was washed with TCA and dissolved in a toluene-soluble quaternary amine (NCS Amersham/Searle, Corp., Arlington Heights, Ill.), and the radioactivity in each sample was determined in a liquid scintillation spectrometer as described previously. An external standard was used for quench corrections.

Results

The proportion of thymocytes bearing the donor type Thy 1 antigen was determined by measuring the inhibition of [^{3}H]TdR incorporation in vitro by specific antiserum and complement as described in the Materials and Methods section. A calibration curve for the assay is shown in Fig 1. AKR/T thymocytes were mixed in known proportions with AKR/C thymocytes, and the percentage of AKR/T cells was then measured using the assay described above. The assay closely estimates the actual proportion of AKR/T cells, although there is a slight underestimate when the proportion exceeds 50%.

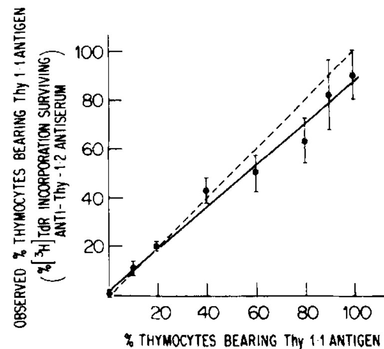

FIG. 1. Calibration of the assay for AKR/T (Thy 1.1) thymocytes in the presence of AKR/C (Thy 1.2) thymocytes. The cells of the two types were mixed in known proportions and the percentage of Thy 1.1 cells was measured by determining the degree of inhibition of [^{3}H]TdR incorporation produced by specific antisera. The solid line indicates the observed results, and the broken line represents the expected results. The vertical bars indicate the standard deviation of the results. The best fit line was determined by least squares regression analysis: $y = 0.84x + 3.13$; $r^2 = 0.991$.

This assay measures only those cells that are synthesizing DNA. Radioautographs made after the infusion of [^{3}H]TdR indicate that this is not an important restriction since the majority of regenerating thymocytes are actively dividing. Thus a single injection of [^{3}H]TdR given on the 12 days after irradiation labels 35–40% of thymocyte nuclei.

Fig. 2 shows the time-course of appearance of cells with the donor type Thy-1 allele in the thymus of irradiated AKR/C hosts (760 R) after injection of either 5 $\times 10^6$ normal AKR/T bone marrow cells or 25 $\times 10^6$ normal AKR/T splenocytes. Cells possessing the donor type Thy 1 antigen were not found until 12 days after irradiation. Thereafter a rapid increase occurred in the proportion of donor type cells among the dividing cells of the thymus, and these are the predominant population by day 20 after irradiation. Spleen cells produced similar results. Several animals were kept for long-term studies. The results indicate that the donor type population is a permanent replacement for the original host type thymocytes, since 200 days after irradiation and repopulation 85 $\pm$ 21% of the cells found in the thymus were of donor origin.

Although mice differing only at the Thy 1 locus are known to reject skin grafts from each other, no evidence of a graft-versus-host reaction was seen in any of the mice studied in these experiments. Thus mice protected with hematopoietic cells bearing the allelic Thy 1 antigen remained chimeric for long periods without signs of wasting or dermatologic lesions. Furthermore, comparisons between the rates of proliferation of injected spleen cells in syngeneic and congenic mice and measurements of spleen size both indicate that cells differing at the Thy 1 locus were neither stimulated nor inhibited by the putatively incompatible environment (Table I). B-cell proliferation in the thymus after injection of the hematopoietic cells from the congenic mice was negligible during the period studied (5–25 days postirradiation), inasmuch as more than 95% of the [^{3}H]TdR incorporation occurred in cells that could be killed by anti-Thy 1 antisera.

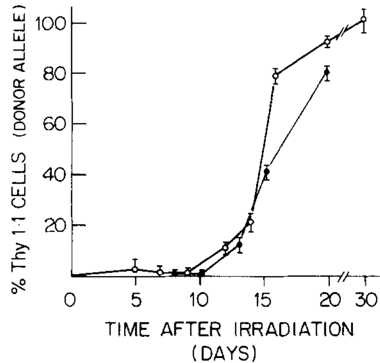

FIG. 2. Time of appearance of cells bearing the donor (Thy 1.1) allele in the thymus of irradiated (760 R) AKR/C (Thy 1.2) mice. (—○—) Percent of Thy 1.1 cells in the thymus after 5×10^6 bone marrow cells. (—●—) Percent of Thy 1.1 cells in the thymus after 2.5×10^7 spleen cells. Four mice/group. The vertical bars indicate the standard deviation at each point.

Effect of Cell Recovery and Amount of [^{3}H]TdR Incorporation on Estimates of the Proportion of Donor Cells in the Thymus. The interpretation of the measurements presented here depends on the fidelity with which the proportion of donor cells in the organ reflects the number of precursor cells injected. Since thymus size is readily altered by outside influences (14, 15) it is necessary to show that these both affect the endogenous (host) thymocytes and those of donor origin equally. Even when irradiation dosage and number of cells transferred are held constant, the extent of thymic regeneration varies considerably from experiment to experiment. Despite this, the proportion of donor cells found at the appropriate time after a fixed dose of transferred cells is remarkably constant. In Fig 3 a and b are shown the data accumulated from 12 experiments performed over an 18-mo period. Cell recoveries varied from 2.2×10^6 to 49.3×10^6 cells/thymus, and these cells were incorporated between 2,800 and 34,500 cpm of [^{3}H]TdR per 200,000 cells under the conditions described above. Except when cell recoveries are extremely poor (less than 5×10^6/thymus) and/or the incorporation of [^{3}H]TdR extremely low (less than 4,000 cpm/200,000 cells) the estimate of the proportion of cells of donor origin is essentially independent of the number of cells recovered and of the rate of DNA synthesis. On the basis of this and similar evidence, data from experiments in which [^{3}H]TdR synthesis falls below 4,000 cpm/200,000 cells were not used. Using the remaining data, we calculate that 10^7 spleen cells contain sufficient thymocyte precursors to give rise to $14.5 \pm 0.3\%$ of the regenerating thymus cells of a 760 R irradiated mouse on the 14th day after transfer (Fig. 3 and 4).

Dose Responses for Bone Marrow and Spleen Cells. The rate of appearance of donor type progeny depends on the number of cells injected. Fig. 4 shows the change in the proportion of donor cells and repopulation indices after varying doses of bone marrow and adult spleen cells. The irradiated AKR/C hosts were injected with cells either 12 or 14 days before their thymuses were examined for donor type progeny. Using the data obtained on day 14 (Fig. 4 b) we calculate that bone marrow contains 8.5 times as many precursors as spleen. Data

TABLE I
Rate of Proliferation of AKR/C and AKR/T Hematopoietic Cells in AKR/C Mice

Source of hemato-poietic cells	Days after transfer	Organ Wt.		$[^3H]$TdR incorporation	
		Spleen	Thymus	Spleen	Thymus
		mg ± SD		*dpm × 10⁻³ ± SD*	
AKR/C	5	30.8 ± 5.8	12.5 ± 1.7	224 ± 102	10.0 ± 7.6
	10	86.5 ± 20.1	14.0 ± 6.0	265 ± 64	33.4 ± 8.3
	15	134.8 ± 39.1	28.5 ± 14.3	129 ± 54	20.3 ± 10.8
AKR/T	5	30.8 ± 30	11.3 ± 0.8	311 ± 149	15.2 ± 7.1
	10	83.7 ± 8.8	20.5 ± 6.2	279 ± 144	57.6 ± 27.3
	15	113.0 ± 62.0	23.2 ± 4.8	125 ± 61	25.1 ± 4.2

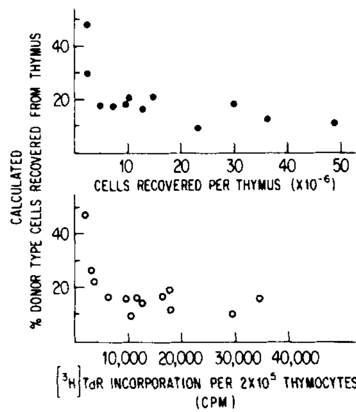

FIG. 3. Influence of the number of cells recovered and the rate of $[^3H]$TdR incorporation by regenerating thymocytes on the proportion of donor type cells in the thymus. Each point represents the results of a separate experiment and is the mean of quadruplicate determinations in pooled thymus cells from three to five mice 14 days after irradiation with 760 R and repopulation with 10^7 spleen cells obtained from 6-10-wk-old AKR/T donors. (3 a above; 3 b below).

obtained on the 12th day in this and in other experiments not shown here, in which larger numbers of spleen cells were used, gives a similar value (7.3). Lymph node cells, on the other hand, produced no detectable thymic repopulation at any dose tested.

The age of the donor mice proved to be a significant source of variation in the degree of repopulation achieved after the injection of constant numbers of hematopoietic cells; this is shown in Table II. The precursor content of the spleen declines precipitiously during the early postpartum period and reaches exceedingly low levels in 10-mo-old mice. In all experiments in which adult bone marrow or spleen cells were transferred, at least 10 days elapsed before donor progeny were detectable in the thymus. In contrast, spleen and bone marrow repopulation occurred promptly, and donor cells were detectable early after irradiation and reconstitution (5, 6). Several experiments were designed to determine the factors that cause the delay in thymus repopulation. The simplest

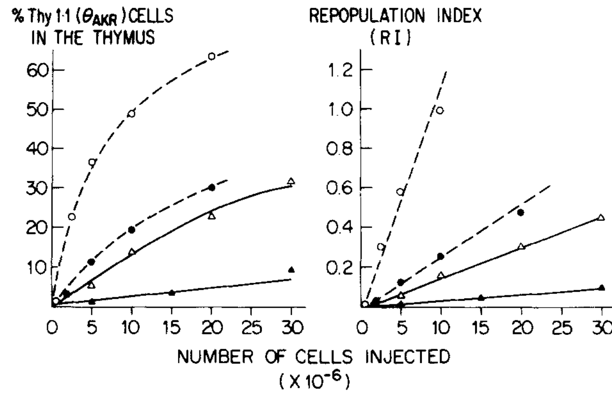

FIG. 4. Proportion of donor type cells formed in the thymuses of AKR/C mice given AKR/T cells. Circles are the results obtained with bone marrow cells while the triangles indicate the results obtained with spleen cells. The data shown by open figures were obtained 14 days, that shown in the solid figures were obtained 12 days after the transfer of donor cells. (a) Percent of donor type cells; (b) repopulation index (4a is the panel on the left).

TABLE II
Age Dependent Changes in Prothymocyte Content of the Spleen

Age	Number of cells injected*	‡ Donor type cells found in thymus (mean ± SD)	§ RI
		%	
Newborn	10×10^6	43.3 ± 2.0	0.76
	3×10^6	30.6 ± 1.1	0.44
	1×10^6	12.5 ± 5.8	0.13
Weanling (25 days)	10×10^6	16.0 ± 0.3	0.19
	3×10^6	6.3 ± 1.2	0.07
Adult (8 wk)	10×10^6	9.9 ± 1.2	0.10
	3×10^6	2.6 ± 0.3	0.03
Old (42 wk)	10×10^6	0.21 ± 0.07	0.0021
	3×10^6	0.17 ± 0.11	0.0017

* AKR/T cells injected into AKR/C mice which had received 760 R γ-radiation.
‡ Examined 14 days after irradiation.
§ Repopulation index calculated as described.

possibility considered was that the delay in appearance of donor type thymocytes after injection of hematopoietic cells was the result of a delay in stem cell entry into the thymus. This could result either from radiation injury to the thymus or a delay in the development of a thymic "homing signal." Postponing the injection of cells until after a hypothesized 'healing' period (or induction period if a homing signal must develop) was over should substantially reduce the delay in the appearance of donor type thymocytes. The effect of postponing

TABLE III
*Appearance of Donor Type Cells in the Thymus After Delayed Bone Marrow Injection**

Marrow cell dose (10^6 cells)	Day 0 injection	Day 5 injection	Day after irradiation
	% donor thymocytes		
10	20.5 ± 4.2	1.9 ± 1.2	12
20	28.4 ± 4.5	1.6 ± 2.0	12
50	ND‡	1.1 ± 2.8	12
10	50.0 ± 7.1	4.8 ± 1.6	15
20	65.8 ± 3.2	5.8 ± 1.0	15

* AKR/C host mice were injected with normal AKR/T bone marrow cells either immediately after irradiation or 5 days later. Cells pooled from the thymocytes of several hosts in each group were assayed for donor type thymocytes 12 and 15 days after irradiation.
‡ ND, not done.

the bone marrow injection for 5 days on the time of appearance of donor type progeny in the thymus are shown in Table III. AKR/C mice were irradiated and given AKR/T bone marrow cells immediately or 5 days later. After 12 and 15 days, the thymuses of these mice were compared. AKR/C hosts which had received marrow soon after irradiation show substantial donor cell proliferation by the 12th day after irradiation and by day 15, more than half of their dividing thymocytes were of donor type. The mice given marrow 5 days after irradiation showed an insignificant number of donor cells on the 12th day and developed only a marginally significant percentage of donor type cells by day 15 (10 days after marrow injection). A substantially larger dose of bone marrow cells on day 5 (50 million cells) was not sufficient to overcome the effect of the 5-day delay in injection. Thus, it appears that delaying the injection of marrow cells delays the appearance of donor type progeny in the thymus. The interval between the injection of bone marrow cells and the appearance of donor progeny in the thymus, however, remains relatively constant.

A more direct test of the accessibility of the irradiated thymic tissue is available. Takada and Takada (5) have reported that some thymocytes "home" promptly to the thymus of irradiated animals. We find that when 10^8 AKR/T thymocytes are injected into irradiated AKR/C mice significant numbers of donor cells are detectable early in the thymus after transplantation. By the 4th day after irradiation 28.0 ± 9.7% of the cells found in the thymus are of donor origin. Thus the delay in the appearance of donor cells after repopulation with hematopoietic cells cannot be due to a physical barrier to cell entry consequent to the irradiation and does not seem to be caused by a late-appearing "homing" signal.

Effect of Irradiation Dose to Recipient on Appearance of Donor Type Cells in the Thymus. One reason for the delay in the appearance of donor cells in the thymus is readily apparent. The indigenous radioresistant precursor population provides a source of thymocytes of host origin. The proliferation of these cells clearly masks the growth of the immigrant donor population. To assess the

TABLE IV
*Effect of Irradiation Dose to the Host on the Percent of Donor Type Cells in the Thymus**

Irradiation dose (rads)	Day of harvest		
	11	14	20
	%	%	%
0 (Normal mice)	2.1 ± 1.2[+]	3.1 ± 1.3	3.0 ± 0.7
125	2.5 ± 0.7	5.9 ± 0.9	2.9 ± 0.3
250	1.6 ± 0.2	2.8 ± 0.5	2.7 ± 0.5
450	2.8 ± 1.7	23.2 ± 1.3	91.9 ± 8.6
700	7.9 ± 1.5	52.9 ± 3.8	98.8 ± 2.9
890	18.9 ± 1.3	73.1 ± 2.9	91.3 ± 2.5

* One Day 0 groups of AKR/C mice received the irradiation doses specified and were injected with 10^7 AKR/T bone marrow cells. On days 11, 14, and 20, after irradiation and marrow injection, cells were pooled from the thymuses of three host mice in each group and assayed for the proporation of cells of donor origin.

extent of this masking, the repopulation experiments were performed at several doses of irradiation. The influence of the radiation dose given to the AKR/C host, on the appearance of donor type thymocytes, is shown in Table IV. All host mice were given 10^7 normal AKR/T bone marrow cells shortly after irradiation, and their thymuses were examined for donor type thymocytes on days 11, 14, and 20 after irradiation. On day 11, only AKR/C hosts which had received 700 R and 890 R before marrow injection had a significant proportion of donor type dividing thymocytes. By day 14, animals that had received 450 R or more had donor type thymocytes. The proportion of these cells is directly related to the dose of irradiation given to the host animal. By day 20 thymuses of host mice which had been exposed to 450 R or more had undergone essentially total replacement of their thymocyte populations with donor type cells. AKR/C host mice which had received 250 R or less continued to show a minimal percentage of donor type thymocytes.

It appears that a minimum level of host cell depletion by irradiation must occur before colonization of the host thymus by donor type stem cells is possible. Table V shows the average cell recovery per thymus of mice that had received 450 R or more. The proportion of donor type cells in each group varies inversely with the number of cells recovered, but as shown in the last column, the absolute number of donor type thymocytes present in each group (calculated by multiplying the proportion donor cells by the appropriate cell yield) appears relatively independent of the dose of irradiation. Thus, the major difference in the regenerating thymuses of mice receiving different irradiation doses is in the size of the proliferating host thymocyte population. Although the size of this population makes detection of the immigrant cells more difficult, it does not account for their late appearance.

Regenerating Hematopoietic Cells as the Source of the Thymocyte Precursor. One possible alternative explanation for the failure of donor cells to gain access to the thymus is that the stem cell simply does not exist in the normal

TABLE V
Cell Recoveries from the Regenerating Thymuses of AKR/C Mice Injected with 10^7 AKR/T Bone Marrow Cells

Day of harvest	γ-Irradiated	Donor type cells (± SD)	Average thymocyte recovery per mouse	Calculated number of donor thymocytes
	rads	%		
11	450	2.8 ± 1.7	151.6×10^6	$4.2 \pm 2.6 \times 10^6$
	700	7.9 ± 1.5	37.3×10^6	$3.0 \pm 0.6 \times 10^6$
	890	18.9 ± 1.3	27.5×10^6	$5.2 \pm 0.4 \times 10^6$
14	450	23.2 ± 1.3	130.3×10^6	$30.2 \pm 1.7 \times 10^6$
	700	52.9 ± 1.3	55.9×10^6	$29.6 \pm 2.1 \times 10^6$
	890	73.1 ± 2.9	50.5×10^6	$36.9 \pm 1.5 \times 10^6$

bone marrow and some differentiative step must occur in the regenerating mouse to produce a cell that can "home" to the thymus. If so, marrow from a lethally irradiated, bone marrow reconstituted mouse (regenerating bone marrow) might be an enriched source of thymic stem cells. To test this, irradiated AKR/C mice were given marrow cells from AKR/T mice which had been lethally irradiated and reconstituted with 5×10^6 normal AKR/T bone marrow cells. The results are shown in Table VI. The irradiated AKR/C mice given normal AKR/T cells show the expected pattern of appearance of donor cells. The AKR/C hosts which had received 7-day regenerating AKR/T marrow showed virtually no entry of donor cells. Thus, 7-day regenerating marrow appears to be depleted of the thymocyte precursor. Similar results were found when either 10-day regenerating marrow or 7-day regenerating spleen was used as the source of donor cells. Circulating cells obtained 3 days after irradiation and bone marrow transfer are also a poor source of thymocyte precursors.

Influence of Transfused Peripheral Cells on Thymic Repopulation. The inability of hematopoietic stem cells to repopulate the thymus early after irradiation might be a consequence of competition among several differentiative pathways for a limited number of multipotential stem cells. Some of these competitive pressures might be ameliorated if adequate numbers of peripheral cells were supplied along with the original hematopoietic cells. To this end large numbers of lymphocytes or erythrocytes were injected into irradiated mice at the same time as the donor bone marrow, and the course of thymic repopulation was studied. The results are shown in Table VII. Neither the administration of 10^8 peripheral lymph node cells nor maintenance of a hematocrit in excess of 60% increased the rate at which donor cells proliferate within the thymus. The lymphocytes in fact appeared to suppress repopulation of the thymus by hematopoietic cells.

Size of the Pool of Cells Entering the Thymus. In view of the preceding it appears that the simplest explanation for the delayed appearance of donor cells in the thymus is that only a small number of donor cells actually enter the organ and that the observed delay reflects the long interval required for these to expand to a readily detectable population. A direct measurement of the number of cells entering the thymus during the first 24 h after irradiation and bone

TABLE VI
Regenerating Hematopoietic Cells as Source of Thymocyte Precursors

Cells injected	Donor type cells in the regenerating thymus (mean ± SD)	
	Day 11	Day 14
	%	
2.5×10^6 normal BM cells	4.1 ± 5.1	24.5 ± 2.5
2.5×10^6 regenerating BM cells	0.3 ± 2.0	−0.4 ± 0.5
5.0×10^6 normal peripheral WBC	ND	3.2 ± 0.3
5.0×10^6 peripheral WBC obtained 3 days postirradiation	ND	0.2 ± 0.2

AKR/C mice were irradiated and then injected with AKR/T cells as indicated. The regenerating marrow donors were AKR/T mice which had received 760 R and 5×10^6 AKR/T BMC 7 days earlier. The peripheral cells were obtained from either normal AKR/T mice or those that had been irradiated and protected with 10^7 BMC 3 days previously. ND, not done.

TABLE VII
Effect of Erythrocyte and Lymphocyte Transfusion on the Repopulation of the Thymus of Irradiated AKR/C Mice

Source of thymocyte precursors	Other cells transferred	(AKR/T) donor type cells in thymus (mean ± SD)*
AKR/T spleen‡	None	21.3 ± 4.2
"	10^8 AKR/C lymphocytes§	12.8 ± 3.6
"	AKR/C erythrocytes‖	19.6 ± 2.8

* Measured 14 days after irradiation using the pooled thymocytes from three to five animals/group.
‡ 2×10^7 cells transferred.
§ Lymphocytes were injected intravenously shortly after the spleen cells. They were pooled, peripheral node cells obtained from the cervical, axillary and inguinal nodes.
‖ 2.0 ml packed erythrocytes were injected intraperitoneally on the day before irradiation.

marrow transfusion was obtained using ^{51}Cr-labeled cells. The results are shown in Table VIII. Assuming uniform labeling of the injected cells, no more than 7,500 cells out of an input of 10,000,000 could have lodged in the thymus during the first 24 h after transfusion.

Time of Entry of Thymocyte Precursors. Although the preceding experiments indicate that only a small number of hematopoietic cells enter the thymus during the first 24 h they do not exclude the possibility that seeding of the thymus with hematopoietic cells occurs continuously from the regenerating marrow rather than directly from the injected marrow. Attempts to isolate the regenerating thymus from further invasion by enclosing reminant from irradiated, bone marrow-protected animals in cell impenetrable chambers have been unsuccessful, but an alternative approach to this problem was attempted. 48 h

TABLE VIII

*Tissue Distribution of ^{51}Cr 24 h after the Injection of ^{51}Cr-Labeled Bone Marrow Cells**

Recipient tissue	cpm + SD‡	Injected radioactivity
		%
Peripheral blood (100 μl)	38.5 ± 9.6	0.031
Thymus	93.3 ± 21.6	0.075
Lung	537.7 ± 124.5	0.431
Liver	33,380 ± 5,211	26.80
Spleen	6,504 ± 1,932	5.21
Femur	1,828 ± 289	1.46

* 10^7 ^{51}Cr-labeled (124,000 cpm/10^7 cells), ACT-treated bone marrow cells were injected into six mice.

‡ cpm are for the entire organ, counted until 10,000 counts had been accumulated and corrected for background. Six individual samples were counted, and the results are given as net arithmetic mean cpm ± SD.

after injecting the congenic hematopoietic cells, a competing dose of syngeneic marrow was administered. If entry is restricted to the immediate postirradiation period only the cells bearing the congenic Thy 1 antigen should be found in the organ. Alternatively, if significant influx from the regenerating hematopoietic tissue occurs, the proportion of cells bearing the congeneic Thy 1 alloantigen should be reduced by dilution with the syngeneic cells. The results of such experiments are shown in Table IX. Only when a large excess of syngeneic precursor cells are injected do they effectively compete with the previously injected congeneic cells and reduce the proportion of congeneic cells found in thymus. Since thymocyte precursors are not detectable in the peripheral circulation during the 1st wk after irradiation and repopulation and since regenerating marrow is also depleted of these cells, we conclude that their entry into the irradiated thymus must occur soon after the injection of the precursor cells.

Discussion

Differences in the Thy 1 alloantigens of otherwise antigenically identical strains of AKR mice have been used to develop a quantitative assay for the extent of chimerism in the thymuses of mice transfused with allogeneic hematopoietic cells after lethal γ-irradiation. The assay is sufficiently sensitive so that as few as 2% cells bearing the donor Thy 1 antigen can be detected. Using this assay we have shown that thymocyte precursors or "prothymocytes" (16, 17) are present in both the bone marrow and spleen of normal mice, but absent from their peripheral lymphoid organs. A similar distribution has been found using an assay that measures the induction of thymic differentiation antigens in previously "null" cells by thymic hormones. Recent evidence presented by Komuro et al. (18) demonstrates that the cells that acquire thymic alloantigens in vitro in the induction assays are in fact capable of entering and proliferating in the irradiated thymus.

TABLE IX
Effect of Competing Syngeneic Cells on the Repopulation of the Thymus by Allogeneic Hematopoietic Cells

Days after irradiation	No. of syngeneic cells injected	Percent Thy 1.1 (donor) cells in regenerating thymus	
		Allogeneic cells only*	Allogeneic cells plus syngeneic cells‡
10		0.2	1.1
14		7.0	9.0
17		21.0	23.5
20		83.0	77.2
14	0	5.8	–
14	3×10^6		6.1§
14	10×10^6		3.8
14	30×10^6		0.9

* 2×10^7 allogeneic (AKR/T) spleen cells.

‡ Allogeneic cells as above. 2×10^6 (ARK/C) bone marrow cells given 48 h later.

§ Allogeneic cells as above. Syngeneic bone marrow cells in the quantities indicated were given 48 h later.

In contrast to B-cell precursors (19), T-cell precursors are more abundant in bone marrow than in adult spleen. Prothymocytes are plentiful in neonatal spleen, but their numbers decline rapidly with age, and old mice have few cells capable of serving as thymocyte precursors. This contrasts with the longevity of B-cell precursors and hematopoietic stem cells which persist throughout the life of the animal (20, 21).

Thymic chimerism, unlike that found in the spleen and bone marrow, develops relatively late after the transfer of the donor hematopoietic cells. There is an interval of 7–10 days during which donor cells are undetectable in the host's thymus. This "eclipse" phase has been noted previously (3, 5, 6, 10) in irradiation-induced chimeras, and qualitatively similar events have been described in thymic grafts in histoincompatible hosts (9, 22–24). Some time in the 2nd wk after the cell transfer a transition period begins during which cells of both donor and host origin are found in the thymus. Ultimately, host cells disappear and only cells derived from the transferred hematopoietic cells are found. This state of chimerism persists throughout the life of the recipient mouse.

The delayed appearance of donor progeny in the thymus appears to be biologically significant since restoration of T-cell mass is thought to be the limiting step in the recovery of immunocompetence after irradiation (24, 25). Since during the transition period the extent of thymic repopulation is directly related to the number of hematopoietic cells transferred, quantitative estimation of thymocyte precursors is possible. Thus we have attempted to delineate the rate-limiting factor(s) in thymic repopulation. The rate of repopulation is

influenced by both the number of precursors available and the dose of irradiation administered. No entry of donor cells into the thymus is detectable when less than 250 R are delivered to the recipient, and between 250 and 450 R the extent of repopulation is somewhat variable. The reason for this is not clear. In the unirradiated mouse, traffic from the bone marrow to thymus is virtually undetectable (3). Repopulation seems to require either the release of a signal from the thymus or depletion of the endogenous stem cell population beyond a critical point. Some thymic regeneration occurs during the "eclipse" phase, and this varies with the dose of irradiation (10). This transitory regeneration is from an intrathymic source and is uninfluenced by transferred hematopoietic cells. The endogenous cells are unable to sustain themselves and disappear from the thymus within 3–4 wk.

Although the size of the residual host population varies with the amount of irradiation delivered, the immigrant population expands at a rate determined by the quantity of hematopoietic cells transferred and independent of the dose of irradiation. Thus the size of the cell population of donor cell origin (Table V) is identical in mice receiving 450, 760, and 890 R. We take this to indicate that for a given number of cells transferred a constant number of stem cells enter the thymus, and this number is independent of the quantity of irradiation received by the host mouse.

A variety of hypotheses could account for the long interval between the induction of chimerism and its development in the thymus. These can be classified into three broad groups. First, there are explanations that postulate that the transferred cells do in fact enter the thymus promptly, but escape early detection; second, there are those that assume that the thymus is in some way unreceptive to migrant cells until some time after irradiation; and finally there are hypotheses that propose that thymic progenitor cells are absent until hematopoietic regeneration has proceeded for some time. We believe the true explanation to be a special case of the first of these, i.e., an extremely small number of prothymocytes enter the receptive thymus almost immediately after the transfer of hematopoietic cells, but are undetectable until they have matured and increased in number. Before discussing the basis for and implications of this explanation, we will briefly consider the evidence against some of the alternatives.

The possibility that substantial numbers of precursor cells enter soon after transfer and remain undetectable for a long period of time can be dismissed promptly. The ^{51}Cr homing data presented here and the autoradiographic analysis presented by Balner and Dersjant (26) indicate that fewer than 0.1% of the injected cells lodge in the thymus and fewer than 1% of the mitoses present 3 days after cell transfer are of donor origin. These do not depend on the expression of thymic differentiation antigens by the infused cells. Only Thy 1-bearing cells are measured in the assay described herein, but by the 5th day postirradiation more than 95% of [^{3}H]TdR incorporation by regenerating thymic cells is susceptible to anti-Thy 1 antisera and complement. Given this and the sensitivity of the assay, it seems highly improbable that substantial numbers of donor cells repopulate the thymus but escape early detection.

The possibility that the delay in repopulation of the thymus is due to an

inaccessibility of the organ is more difficult to eliminate. The fact that thymocytes injected soon after irradiation gain access and multiply in sufficient number as to make up one-fourth of the dividing cells 5 days after irradiation rules out simple radiation injury as a bar to cellular immigration, but does not eliminate the possibility that entry requires the appearance or change in magnitude of a "homing" signal generated in the gland. This presumably would occur after the endogenous precursor population was depleted. A clear example of such a signal has been presented by LeDouarin and Jotereau (27) who studied the entry of stem cells into the embryonic avian thymus. If, however, such were the case, we would expect that delaying the infusion of donor marrow would not produce a proportionate delay in the appearance of progeny in the thymus, since the interval between the development of the "homing" signal and the appearance of donor progeny, rather than the time between infusion and appearance, should be the controlling factor. Our data indicate that this is not so.

The irradiated thymus would also appear inaccessible if it contained an extremely small number of sites to which stem cells could "home". Although the precise number of such sites cannot yet be estimated it is evident that they exceed the number of stem cells circulating after infusion of hematopoietic cells, since the extent of repopulation is directly proportional to the number of infused cells, and saturation of the available sites was not attained at any of the tested doses of hematopoietic cells.

The alternative possibility that the true precursor cells are absent from the infused hematopoietic cells and require time to differentiate into cells capable of repopulating the thymus also appears unlikely since regenerating marrow and spleen, sources of cells that might be expected to be enriched in such cells, are in fact depleted of them. Progenitor cells are also absent from the circulation at the time when any theory of late development or late entry requires their presence. We can not exclude the possibility that entry of the precursor cells requires some alteration in their cell surface, perhaps induced by a humoral signal from the thymus, but if so, neither of these processes appear to be rate limiting. It might be argued that if a multipotential stem cell was the immediate precursor of the thymic lymphocyte, competing requirements for differentiation into the B-cell or erythroid compartments might effectively deplete the infused cells of their capacity to repopulate the thymus and also explain the paucity of precursors found in regenerating marrow. The likelihood that competition of this sort is an adequate explanation for the delayed appearance of donor progeny seems small since infusion of huge numbers of peripheral lymphocytes and large numbers of mature erythrocytes failed to accelerate the repopulation of the thymus by the AKR/T hematopoietic cells.

The evidence for our idea that repopulation occurs from a small number of progenitor cells which enter soon after their infusion can be summarized briefly. A small number of cells do enter. This number is less than 0.1% of injected bone marrow cells, but is independent of the dose of irradiation delivered to the recipient. The time at which these cells can first be detected is related to both the number of progenitors infused and also the number of residual endogenous cells. Increasing the dose of radiation does reduce the number of syngeneic cells found in the thymus (10) and does, as shown in Table IV, permit earlier detection of the donor cells. Comparing the number of donor type cells found in

the thymus at different times after transplantation at several doses of irradiation allows us to make a minimal estimate of the rate of increase of this population. Cell death and/or emigration are ignored in such a calculation. In the 72 h between days 11 and 14 the number of donor type thymocytes found in mice that had received 10^7 bone marrow cells increased by 26 million after 450 R, 27 million after 700 R, and 32 million after 890 R. Assuming that these increases are the result of exponential growth of thymus-entering cells and no egress occurred during the interval, a doubling time of 24 h can be calculated. This is far slower than the 8.5–10 h doubling times reported for normal cortical thymocytes (28, 29), but extrapolation of even this slow rate back to the time of transfer indicates that the cells present on the 11th day are the progeny of 150–200 cells lodging in the thymus out of each million transferred.

This is consistent with our estimates of the number of cells actually entering the gland (Table VIII). A multitude of assumptions are involved in such a calculation. Most of them tend to overestimate the doubling time and therefore overestimate the number of progenitor cells required to achieve the observed results. Cytokinetic data on regenerating thymus indicate that the regenerating cortical cells observed on the 14th day after irradiation have a doubling time of 10–12 h.[2] If this rate were to persist throughout the regeneration period only one cell would be sufficient to produce all of the donor progeny found on the 11th day after the transfer of 10 million bone marrow cells. This is certainly an underestimate and suggests that the regenerating cells go through a phase during which they proliferate relatively slowly. A similar conclusion has been reached by Decleve et al. (30). The detection of the progeny of a small number of slowly dividing cells amidst the proliferating endogenous population poses nearly insurmountable problems. It is both the failure of the endogenous population to continue its growth and a change in the mitotic rate of repopulating cells that eventually permit the expanding donor-type thymocyte population to manifest itself.

If these estimates are correct they raise interesting questions as to the timing of the acquisition of immunologic diversity among "T" lymphocytes. Full hematopoietic reconstitution of irradiated mice can be achieved with as few as 10^6 bone marrow cells (24, 25, 31). If the prothymocytes in this population are clonally restricted in their immunologic potential before they enter the gland then only a small number of specificities should be recognized by the early emerging T cells. If, however, diversity is generated during the thymic sojourn, an ample number of cells could be generated within the 2–3-wk period required for thymic regeneration. These possibilities can be distinguished experimentally. The results should indicate whether or not the events that lead to the generation of immunologic diversity can occur during adult life or are restricted to a specific phase of embryonic development.

Summary

A quantitative assay for the hematopoietic precursor of thymocytes has been developed. Using this assay the kinetics of appearance of the progeny of trans-

---

[2] R. S. Basch, and J. Kadish. Unpublished results.

fused bone marrow and spleen cells in the thymus of irradiated (760 R) mice has been studied. Precursor cells are seven to eightfold more common in bone marrow than in spleen and are absent from peripheral lymph nodes. They decline in number as the animals age. When hematopoietic cells are injected immediately after lethal irradiation only a small number of cells actually enter the gland. Their progeny are not detectable in the thymus for 8–12 days. The time of their detection depends both upon the size of the residual endogenous thymocyte population and the number of progenitor cells injected. Evidence has been presented that excludes thymic injury as the basis for the delay in the appearance of donor type cells and indicates that neither the production of a "homing" signal in the irradiated animal nor the development of precursor cells are limiting factors in the rate of thymic repopulation.

These studies indicate that only an exceedingly small number (less than 100) of prothymocytes are required to repopulate the thymus of an irradiated mouse. This restricted number of progenitors must produce the entire repertory of T-cell immunologic responsiveness seen in the first weeks after repopulation.

The excellent technical skills of Bohumila Fahmy, Shirley Carchi, and Thomas Panagiotatos greatly facilitated this work. Dr. H. Lustig assisted with the autoradiography. Discussions with Doctors J. Phillips-Quagliata and M. Lamm were most helpful.

Received for publication 10 December 1975.

References

1. Badertcher, J. A. 1915. The development of the thymus in the pig. *Am. J. Anat.* 17:317.
2. Harris, J. E., D. W. H. Barnes, C. F. Ford, and E. P. Evans. 1964. Cellular traffic of the thymus: experiments with chromosome markers. Evidence from parabiosis for an afferent stream of cells *Nature (Lond.)*. **201**:886.
3. Ford, C. E., H. S. Micklem, E. P. Evans, J. G. Gray, and D. A. Ogden. 1966. The inflow of bone marrow cells to the thymus: studies with part body irradiated mice injected with chromosome marked bone marrow and subjected to antigenic stimulation. *Ann. N. Y. Acad. Sci.* **129**:283.
4. Gengozian, N., I. S. Urso, C. C. Congdon, A. D. Conger, and T. Makinodan. 1957. Thymus specificity in lethally irradiated mice treated with rat bone marrow. *Proc. Soc. Exp. Biol. Med.* **96**:714.
5. Takada, A., and Y. Takada. 1973. Proliferation of donor marrow and thymus cells in the myeloid and lymphoid organs of irradiated syngeneic host mice. *J. Exp. Med.* **137**:543.
6. Hiesche, K. D., and L. Revesz. 1974. Cellular repopulation in irradiated mouse thymus and bone marrow. *Beitr, Pathol.* **151**:304.
7. Order, S. E., and B. H. Waksman. 1969. Cellular differentiation in the thymus. *Transplantation (Baltimore)*. **8**:783.
8. Moore, M. A. S., and J. J. T. Owen. 1967. Experimental studies on the development of the thymus. *J. Exp. Med.* **126**:715.
9. Owen, J. J. T., and M. C. Raff. 1970. Studies on the differentiation of thymus-derived lymphocytes. *J. Exp. Med.* **132**:1216.
10. Kadish, J. L., and R. S. Basch. 1975. Thymic regeneration after irradiation: evidence for an intra-thymic radioresistant T cell precursor. *J. Immunol.* **114**:452.
11. Acton, R. T., E. P. Blankenhorn, T. C. Douglas, R. D. Owen, J. Hilgers, H. A.

Hoffman, and E. A. Boyse. 1973. Variations among sublines of inbred AKR mice. *Nature New Biol.* **245**:8.

12. Wigzell, H. 1965. Quantitative titrations of mouse H-2 antibodies using ^{51}Cr labeled target cells. *Transplantation (Baltimore).* **3**:423.
13. Boyle, W. 1968. An extension of the ^{51}Cr-release assay for the estimation of mouse cytotoxins. *Transplantation (Baltimore).* **6**:761.
14. Boyd, E. 1932. The weight of the thymus in health and disease. *Am. J. Dis. Child.* **43**:1162.
15. Gad, P., and S. L. Clark Jr. 1968. Involution and regeneration of the thymus induced by bacterial endotoxin and studied by quantitative histology and electron microscopy. *Am. J. Anat.* **122**:573.
16. Basch, R. S., and G. Goldstein. 1974. Induction of T-cell differentiation *in vitro* by thymin, a purified polypeptide hormone of the thymus. *Proc. Natl. Acad. Sci. U.S.A.* **71**:1474.
17. Komuro, K., and E. A. Boyse. 1973. *In vitro* demonstration of thymic hormone by the mouse by conversion of precursor cells into lymphocytes. *Lancet.* **1**:740.
18. Komuro, K., G. Goldstein, and E. A. Boyse. 1975. Thymus repopulating capacity of cells that can be induced to differentiate into T cells *in vitro*. *J. Immunol.* **115**:195.
19. Lafleur, L., R. G. Miller, and R. A. Phillips. 1973. Restriction of specificity in the precursors of bone marrow associated lymphocytes. *J. Exp. Med.* **137**:954–966.
20. Harrison, D. E., and J. W. Doubleday. 1975. Normal function of immunologic stem cells from aged mice. *J. Immunol.* **114**:1314.
21. Harrison, D. E. 1973. Normal production of erythrocytes by mouse marrow continuous for 73 months. *Proc. Natl. Acad. Sci. U.S.A.* **70**:3182.
22. Dukor, D., J. F. A. F. Miller, W. House, and V. Allman. 1965. Regeneration of thymus grafts I. Histological and cytological aspects. *Transplantation (Baltimore).* **3**:639.
23. Cheers, C., E. Leuchars, V. Wallis, and A. J. S. Davies. 1972. Cell population changes in regenerating thymus grafts. *Transplantation (Baltimore).* **13**:72.
24. Chiscon, M. O., and E. S. Golub. 1972. Functional development of the interacting cells in the immune response. *J. Immunol.* **105**:1379.
25. Blomgren, H., and B. Anderson. 1971. Appearance and relative importance of immunocompetent cells in the thymus, spleen and lymph nodes following lethal X-irradiation. *J. Immunol.* **106**:831.
26. Balner, H., and H. Dersjant. 1964. Early lymphatic regeneration in thymectomized radiation chimeras. *Nature (Lond.).* **204**:941.
27. LeDouarin, N. M., and F. V. Jotereau. 1975. Teasing of cells of the avian thymus through embryonic life in interspecific chimeras. *J. Exp. Med.* **142**:17.
28. Fabrikant, J. I., and B. R. Foster. 1972. Cell population kinetics in lymphocytes in mouse and rat thymus. *Johns Hopkins Med. J.* **130**:208.
29. Bryant, B. J. 1972. Renewal and fate in the mammalian thymus: mechanisms and influences of thymocytokinetics. *Eur. J. Immunol.* **2**:38.
30. Decleve, A., G. B. Gerber, A. Leonard, M. Lambiet-Collier, A. Sassen, and J. R. Maisin. 1972. Regeneration of thymus, spleen, and bone marrow in X irradiated AKR mice. *Radiat. Res.* **51**:318.
31. Rubin, B., L. Trier, B. Hertel-Wulff, and B. Rumle. 1975. Selective retention of T cell precursors in bone marrow cell populations after filtration through anti-immunoglobulin columns. *Cell. Immunol.* **16**:315.