

THE LIFE-SPAN AND RECIRCULATION OF MARROW-
DERIVED SMALL LYMPHOCYTES FROM THE RAT
THORACIC DUCT

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It is known that peripheral lymphoid tissues become populated by dividing, marrow-derived cells in thymectomized, irradiated animals reconstituted with normal marrow (1). Partial repopulation of lymphoid tissue has also been observed histologically after neonatal thymectomy (2, 3) and after adult thymectomy followed by sublethal irradiation (4) and many of the repopulating cells are small lymphocytes, although their marrow origin has not been directly demonstrated. The importance of marrow-derived lymphocytes has been emphasized by the discovery that they provide the precursors of antibody-forming cells in mice (5) but uncertainty remains about their morphological identity and life history.

Experiments in rats have shown that the precursors of antibody-forming cells are normally present among the small lymphocytes in the thoracic duct (6, 7), and in thymectomized radiation chimeras these lymph-borne precursors have been shown to be marrow derived.¹ The aims of the present study were (a) to demonstrate formally that marrow can give rise to small lymphocytes which enter the thoracic duct and complete their differentiation in the absence of the thymus; (b) to determine if the life-span of these small lymphocytes is short, characteristic of the majority of small lymphocytes of marrow from which they came (8, 9), or long, characteristic of the majority of peripheral small lymphocytes which they have joined (10-12); and (c) to determine whether they recirculate from blood to lymph.

Materials and Methods

Animals.—Young adult male and female rats of the inbred HO strain were used in syngeneic transfer experiments, and (HO × AO)F₁ and (HO × DA)F₁ hybrids as marrow donors in semiallogeneic transfers to demonstrate the marrow origin of peripheral cells.

Operative Procedures.—Thoracic duct cannulation and adult thymectomy were performed as described previously.²

¹ Scott, D. W., and J. C. Howard. Collaboration between thymus-derived and bone marrow-derived thoracic duct lymphocytes in the hemolysin response of the rat. *Cell Immunol.* In press.

² Howard, J. C., and D. W. Scott. The role of recirculating lymphocytes in the immunological competence of rat bone marrow cells. *Cell Immunol.* In press.

Irradiation.—2–4 wk after thymectomy, prospective bone marrow recipients received 1000 rads whole body γ -irradiation from a ^{60}Co source at a dose rate of 150 rads/min. Recipients were given Terramycin (Pfizer, Ltd., Sandwich, England) in the drinking water at a concentration of 9 g/liter (tetracycline concentration, 450 mg/liter) from the day before irradiation until the end of the experiment.

Marrow Cell Suspensions.—In some experiments marrow suspensions were prepared from the femurs and tibias of normal 10–12-wk-old male donors, and injected in doses of 200×10^6 viable cells. In the remaining experiments, marrow was obtained from donors which had been thymectomized 2 wk previously, and drained of recirculating lymphocytes from a chronic thoracic duct fistula for 7 days up to the day of killing. This procedure severely depletes the population of recirculating thymus-derived lymphocytes normally present in rat marrow.² A dose of 10^7 viable marrow cells from lymphocyte-depleted donors was used to reconstitute irradiated recipients.

Marrow suspensions were prepared at 4°C in Dulbecco's buffered salt solution (Oxoid Ltd., London) containing 2% fetal calf serum (DAB/FCS).³ Viability assayed by trypan blue exclusion invariably exceeded 80%. Suspensions were injected via the lateral tail vein on the same day as irradiation.

Cytotoxic Alloantisera.—Four cytotoxic alloantisera were used to test for lymphocyte alloantigens: HO-anti-DA and HO-anti-AO raised in multiparous HO females mated to DA or AO males as described previously (7); and DA-anti-HO and AO-anti-HO raised by repeated immunization of DA and AO rats with pooled lymphoid cells from HO donors. Cytotoxic tests were performed on freshly collected thoracic duct lymphocytes (TDL) as described previously.¹

Labeling of TDL In Vitro.—TDL were washed in DAB/FCS at 4°C and resuspended at 5×10^7 cells/ml in Dulbecco modified Eagles medium with 10% FCS and tryptose phosphate broth. Uridine-5- ^3H (5 Ci/mM), and in some cases also thymidine-2- ^{14}C (48 mCi/mM) (Radiochemical Centre, Amersham, England) were added to a final concentration of 5 $\mu\text{Ci/ml}$. Suspensions were incubated in glass bottles for 75 min at 37°C in an atmosphere of 5% CO_2 in air, and cells were gently resuspended by shaking every 15 min. After labeling, cells were washed three times in 10 ml volumes of DAB/FCS at 4°C and finally resuspended in DAB/FCS for injection through established femoral vein cannulae (see below) at a cell concentration of $50\text{--}100 \times 10^6/\text{ml}$.

Labeling of TDL In Vivo.—Thymidine-6- ^3H (5 Ci/mM, Radiochemical Centre) was infused into the femoral vein of restrained rats at the rate of 1 $\mu\text{Ci/g}$ body weight per 24 hr, at a flow rate of 2.3 ml/hr, for periods of 36 hr or 7 days.

Radioautography.—Smears were fixed in methyl alcohol, extracted 3 times for 5 min each in 5% trichloroacetic acid (TCA) at 4°C, washed in running tap water and in distilled water. After drying, slides were dipped in Ilford K5 emulsion (Ilford Ltd., Ilford, Essex, England) and exposed to obtain optimal labeling. After development the slides were washed in tap water for 30–60 min followed by distilled water and stained with Giemsa at $1/10$ in distilled water for 35 min, followed by prolonged differentiation in distilled water.

Counting Labeled Cells on Radioautographs.—Distributions of background grains over 1000 small lymphocytes counted on radioautographs prepared from unlabeled thoracic duct cells conformed closely to modified Poisson distributions. In experimental radioautographs >90% of small lymphocytes carried grains due only to background, but the observed distributions of grain counts over 1000 cells departed significantly from those due to background alone due to the presence of truly labeled cells. All small lymphocytes with more than 10 grains were counted as labeled since the mean grain count for all other small lymphocytes in no case exceeded 1.6. The number of truly labeled cells with 10 grains or fewer was estimated for each

³ Abbreviations used in this paper: DAB-FCS, Dulbecco's buffered salt solution containing 2% fetal calf serum; TDL, thoracic duct lymphocytes.

radioautograph by removing cells with the highest grain counts from the observed distribution one by one and calculating a modified Poisson distribution with the mean and variance of each residual observed distribution. A conservative estimate of the number of labeled cells was obtained when the grain count distribution of residual cells did not depart significantly from a modified Poisson distribution at the 5% level. This procedure provides an internal correction for fluctuations in the level of background grains over cells on experimental radioautographs and tends to underestimate the frequency of labeled cells, since increases in background mean and variance lead to reductions in the estimated frequency of labeled cells.

Scintillation Counting.—Labeled lymphocyte suspensions for injection were washed at 4°C in DAB/FCS, and 0.1-ml aliquots at a known cell concentration transferred to counting vials. Lymphocytes from the thoracic duct of animals receiving labeled cell inocula intravenously were washed once and spun down in disposable plastic centrifuge tubes. Cell suspensions or pellets were prepared for scintillation counting (13), and counted in a Beckman LS-250 liquid scintillation spectrometer (Beckman Instruments, Inc., Fullerton, Calif.).

RESULTS

Identification of Marrow-Derived Small Lymphocytes.—Marrow-derived lymphocytes were obtained by submitting young adult rats to the following procedures in succession: (a) thymectomy; (b) 1000 rads gamma irradiation 2–4 wk after thymectomy followed on the same day by (c) reconstitution with dissociated bone marrow cells; (d) a period of not less than 3 wk to allow marrow-derived cells to differentiate and peripheralize; (e) collection of lymphocytes from the thoracic duct. Rats subjected to these procedures are referred to as B rats.

Table I gives the cell composition of lymph from B rats 4, 8, and 12 wk after their restoration with 2×10^8 marrow cells from normal syngeneic donors. The total lymphocyte output during the first 12 hr of thoracic duct drainage was 20–30% of normal, and differential counts on smears and on fresh cells

TABLE I
Output of Lymphocytes from Thoracic Duct of Normal HO Rats and of Thymectomized, Irradiated HO Rats Reconstituted with 2×10^8 Normal Marrow Cells (B Rats)

	Lymphocyte output ($\times 10^6$ /hr)*		After marrow reconstitution	No. rats
	Small	Large		
			<i>wk</i>	
B rats	5.05 (1.98–9.27)	1.77 (0.97–3.63)	4	17
	6.53 (4.01–10.0)	2.27 (1.41–3.16)	8	10
	8.49 (5.27–11.0)	1.81 (1.23–2.47)	12	6
Normal rats	26.6 (16.0–40.5)	1.43 (0.4–3.3)	—	23

* Mean and range from first 12 hr collection after thoracic duct cannulation.

in counting chambers showed the deficit to be exclusively among the small lymphocytes.

All the lymphocytes in the thoracic duct of B rats were shown to be derived from marrow in experiments in which thymectomized, irradiated HO rats were restored with 2×10^8 marrow cells from (HO \times AO) F_1 or (HO \times DA) F_1 donors. The total and differential lymphocyte counts on the lymph of B rats 5 and 8 wk after restoration with F_1 hybrid marrow were in the same range as those recorded in Table I for the groups given syngeneic marrow. Cytotoxic assays with alloantisera showed that in each of the donor-recipient combinations the thoracic duct lymphocytes of the B rats carried the unshared antigens

TABLE II
Marrow Origin of Thoracic Duct Lymphocytes (TDL) from B Rats

TDL donors	Treatment of TDL with alloantisera				
	Dead cells* with				
	HO-anti- AO	AO-anti- HO	HO-anti- DA	DA-anti- HO	
			%		
Normal rats	HO	1	99	2	100
	(HO \times AO) F_1	92	97	—	—
	(HO \times DA) F_1	—	—	100	100
B rats‡	(HO \times AO) F_1 \rightarrow HO (55d)	91	96	—	—
	(HO \times DA) F_1 \rightarrow HO (34d)	—	—	100	100

* Based on 180–240 cells counted in 0.5% trypan blue.

‡ Thymectomized, irradiated HO rats received 2×10^8 marrow cells from (HO \times AO) F_1 or (HO \times DA) F_1 donors 55 and 34 days, respectively, before thoracic duct cannulation.

of the marrow donor (Table II). No evidence was obtained for a host-derived population of cells in the thoracic duct.

Although the results in Table II show clearly the marrow origin of the small lymphocytes in lymph from B rats, they do not exclude an origin from the thymus-derived, recirculating lymphocytes which are normally present in rat marrow² and which would have “contaminated” the restorative marrow inocula.

Thymus-derived cells have been detected sporadically by immunological methods in the thoracic duct of B rats reconstituted with 2×10^8 normal marrow cells,¹ but further results showed that even when present, thymus-derived cells must be rare. Thymectomized, irradiated rats were reconstituted with small marrow inocula (10^7 cells/recipient) from rats severely depleted of recirculating, thymus-derived lymphocytes by thymectomy and chronic thoracic duct drainage (see Materials and Methods). No thymus-derived lymphocytes have been detected among thoracic duct cells from such B rats,¹ yet the cellular composition of their lymph and their small lymphocyte outputs were indistinguishable from those of B rats receiving 2×10^8 normal marrow cells (Fig. 1).

In B rats receiving 10^7 lymphocyte-depleted marrow cells, a single 12 hr lymphocyte collection amounted to between 5 and 15 times the total marrow inoculum, so there is no doubt that the small lymphocytes in the thoracic duct were derived from a proliferative source in the marrow. The fact that the small lymphocyte outputs were the same in the two types of B rat pointed to this proliferative source as a generator of true marrow-derived lymphocytes in both cases. In subsequent experiments on the life-span and recirculation of marrow-derived cells, both types of B rat were used as lymphocyte donors, and in each case the same conclusion could be drawn: the frequency of thymus-

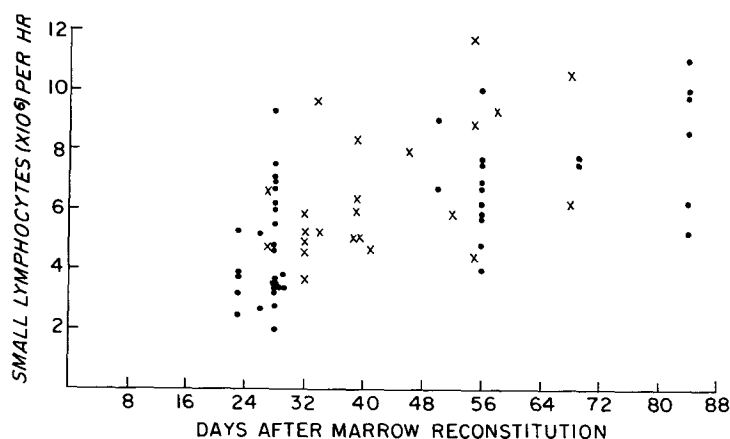


FIG. 1. Thoracic duct small lymphocyte outputs in thymectomized, irradiated rats reconstituted with either 2×10^8 normal marrow cells (●) or 10^7 lymphocyte-depleted marrow cells (X). Each point represents the output of one rat during the first 12-hr lymph collection after thoracic duct cannulation.

derived cells in the lymph of B rats reconstituted with 2×10^8 normal marrow cells was too low to be detected (see Table III, Figs. 2, 3).

Longevity of Marrow-Derived Small Lymphocytes.—Estimates of lymphocyte “life-span” have been made from measurements of the rate at which labeled cells accumulate in or disappear from a particular anatomical compartment (e.g. blood, lymph, or nodes) during or after the administration of thymidine- ^3H . The point to be determined in the present experiments is whether the small lymphocytes in the lymph of B rats correspond with respect to life-span to the short-lived (5–7 days) or the long-lived (>10 days) populations described for normal rats (11, 12). An association between marrow-derivation and short life-span of small lymphocytes has been postulated (14).

Tritiated thymidine was infused intravenously into B rats and normal rats in two regimens intended to label large lymphocytes (36 hr infusion) and short-lived small lymphocytes (7 days infusion). Thoracic ducts were cannu-

lated during the last day of infusion and cells collected for radioautography over the final 12 hr period.

Table III shows that the patterns of labeling of thoracic duct lymphocytes from normal and B rats were very similar. Virtually all large lymphocytes were labeled in both populations by 36 hr, while the two periods of infusion labeled small but increasing proportions of small lymphocytes. Moreover, as stated

TABLE III
Accumulation of Labeled Lymphocytes in Thoracic Duct of Normal and B Rats after Continuous Infusions of Thymidine-³H

Thymidine- ³ H infusion	TDL donors	Labeled lymphocytes*			Mean total labeled per cent
		Small	Large	Total	
36 hr	B† (a)	1.9	78.2	30.2	30.4
		0.9	83.1	27.6	
		2.4	75.0	33.5	
	Normal	2.2	79.2	4.3	6.1
		3.0	88.7	7.6	
		2.4	80.3	6.5	
7 days	B† (a)	9.5	97.7	54.6	44.7
		9.8	98.2	50.9	
		13.1	99.0	34.6	
		11.2	97.4	38.8	
	Normal	4.6§	96.6	10.5	11.5
		3.0§	90.0	8.5	
		10.1	97.7	12.7	
		11.4	100.0	14.2	

* Smears made from thoracic duct lymphocytes collected during the last 12 hr of the infusion period. 1000 cells counted per rat.

† B rats were reconstituted with either 2×10^8 normal marrow cells (a) or 10^7 lymphocyte-depleted marrow cells (b).

§ These two figures are unexpectedly low for the frequency of labeled thoracic duct small lymphocytes after 7 days administration of thymidine-³H to normal rats (15, 16).

above, there were no systematic differences between lymphocyte populations from the two types of B rats.

The results in Table III are open to the criticism that it is impossible to classify rigidly all lymphocytes as either "small" or "large." This criticism can be answered in the present context by considering the frequency of all labeled lymphocytes. It can be seen that there is a substantial excess of labeled cells in the B rats both at 36 hr and 7 days. Nevertheless, the increment of total labeled cells over the interval between 36 hr and 7 days is small in the B rats, and of the same order as that found in normal rats, showing that B rats

do not possess an unusually high proportion of any type of lymphocyte with a life-span between 36 hr and 7 days. From these results it seems that the life-span of the majority of marrow-derived small lymphocytes is similar to that of normal small lymphocytes; certainly there is no evidence that they are predominantly short-lived.

The Recirculation of Marrow-Derived Lymphocytes from Blood to Lymph.—In view of the long life-span of the majority of the marrow-derived small lymphocytes in the thoracic duct of B rats, it seemed likely that they would be found to recirculate between blood and lymph. This point was studied by determining the capacity of small lymphocytes from the thoracic duct of B rats to reenter the lymph after injection into the blood. A preliminary experiment established that labeled lymphocytes from B rats did, in fact, appear in the thoracic duct lymph after intravenous injection and that recirculation could be demonstrated equally well whether the recipients were normal or B rats.

The first overnight collections of TDL from two B rats reconstituted with 2×10^8 normal marrow cells and from two normal (N) rats were labeled *in vitro* with uridine- ^3H . The labeled cells were then returned to the cannulated donors through the femoral vein according to the symmetrical plan shown in Fig. 2. Cell-associated radioactivity was counted in recipient thoracic duct lymph between 14 and 24 hr after cell injection, a period which includes the mode of recovery of intravenously injected normal cells in normal recipients (17). It is clear from Fig. 2 that the amount of label recovered depended upon the source (B or N) of the injected cells, and not upon the identity of the recipient, suggesting that lymphocytes were not found in the thoracic duct of B rats simply because their vascular structures were abnormal. It is also clear that recovery of radioactivity as a function of injected dose was much lower with lymphocytes from B rats than with lymphocytes from normal donors. This showed that some marrow-derived lymphocytes recirculated from blood to lymph, but suggested that they did so with a different tempo from that of normal lymphocytes.

Recirculation of Marrow-Derived TDL Shown by Scintillation Counting: Effect of Large Lymphocytes on Estimated Recirculation.—Subsequent experiments examined in detail the tempo of recovery of label from the thoracic duct after intravenous injection into normal recipients of labeled marrow-derived thoracic duct lymphocytes. Lymphocytes were collected from the thoracic duct of B rats between 4 and 8 wk after reconstitution with 10^7 lymphocyte-depleted marrow cells, labeled with uridine- ^3H , and transfused intravenously into normal syngeneic recipients bearing an established thoracic duct fistula. In each experiment the recirculation of lymphocytes from B donors was compared with the recirculation of labeled lymphocytes from normal donors in similar recipients. Thoracic duct lymphocytes were collected from all recipients in consecutive 3-hr pools and prepared for scintillation counting every 12 hr. The outputs of cell-associated radioactivity from recipients of normal and

marrow-derived lymphocytes are presented as a percentage of injected radioactivity in Fig. 3. Substantial quantities of cell-associated label recirculated from blood to lymph in recipients of marrow-derived lymphocytes. The mean total output of radioactivity over a 48 hr period was 5.4% of the injected dose against 16.9% of injected dose for lymphocytes from normal donors. The

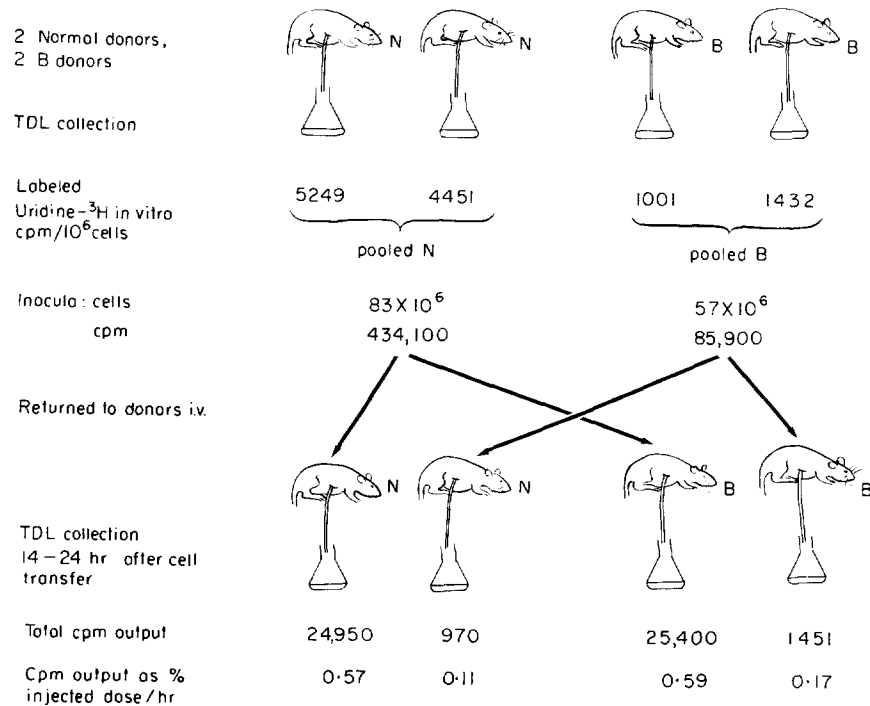


FIG. 2. Recirculation of normal and marrow-derived thoracic duct lymphocytes. Preliminary experiment to establish that the recirculation of marrow-derived cells was different from normal cells and that this difference was an intrinsic property of the cells, not of the recipient. Note the deficient uridine uptake by marrow-derived lymphocytes.

tempo of recovery of radioactivity associated with marrow-derived lymphocytes was characterized by a 12 hr mode compared with the 15-24 hr mode of recovery for normal lymphocytes. It is not valid to equate recovery of radioactivity directly with recovery of small lymphocytes. The 20-30% large lymphocytes in an inoculum of marrow-derived lymphocytes account for as much as 70% of the total radioactivity injected, while the 5% of large lymphocytes in a normal lymphocyte suspension make a negligible contribution to the total label. This important discrepancy is due both to the high frequency of large lymphocytes in B lymphocyte suspensions and to the fact

that marrow-derived small lymphocytes show a striking deficit of uridine uptake in vitro compared with thymus-derived small lymphocytes (18).

Large lymphocytes in S phase in freshly collected lymph, which therefore

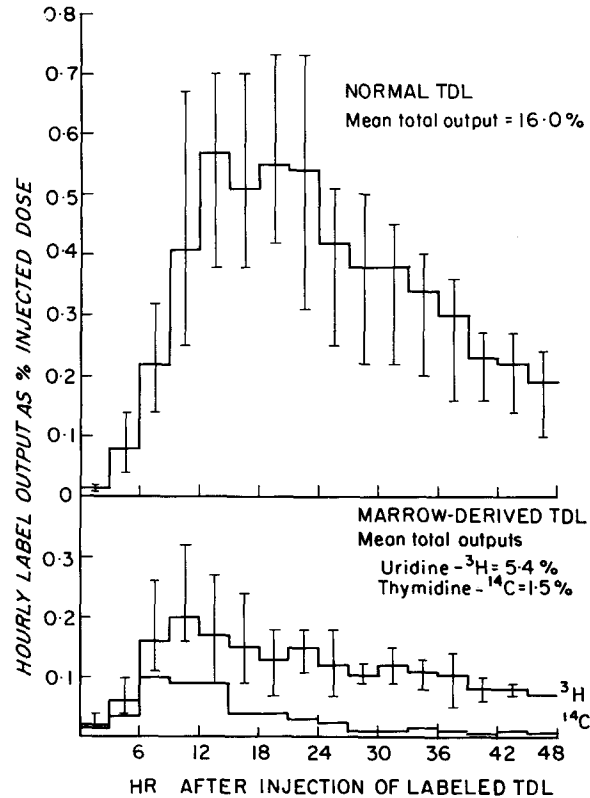


FIG. 3. Recirculation of normal and marrow-derived thoracic duct lymphocytes (TDL) in normal recipients. Normal TDL were labeled with uridine- ^3H only (four expts); marrow-derived TDL were labeled with uridine- ^3H only (two expts) and both uridine- ^3H and thymidine- ^{14}C (two expts). Labeled cells were transferred intravenously to normal recipients bearing thoracic duct fistulae. Cell-associated radioactivity was counted in 3-hr pools of lymph from injected recipients and expressed as the output of label (^3H or ^{14}C) from the thoracic duct per hour as a percentage of the total injected dose of label (^3H or ^{14}C). Histograms and limits represent means and ranges of outputs.

incorporate thymidine in vitro, also incorporate disproportionately large amounts of uridine in vitro (18, 19). Consequently the recovery of radioactivity from the recipient thoracic duct after transfer of uridine-labeled marrow-derived cells is substantially dependent on the extent to which these high uridine uptake large lymphocytes reenter lymph from the blood. The next experiments therefore recorded for marrow-derived thoracic duct cells the

magnitude and tempo of S phase large lymphocyte recirculation and determined the relative contributions of large and small lymphocytes to the observed recovery of radioactivity.

Thoracic duct lymphocytes collected from B rats 46 days after reconstitution with 10^7 lymphocyte-depleted marrow cells, and labeled in vitro simul-

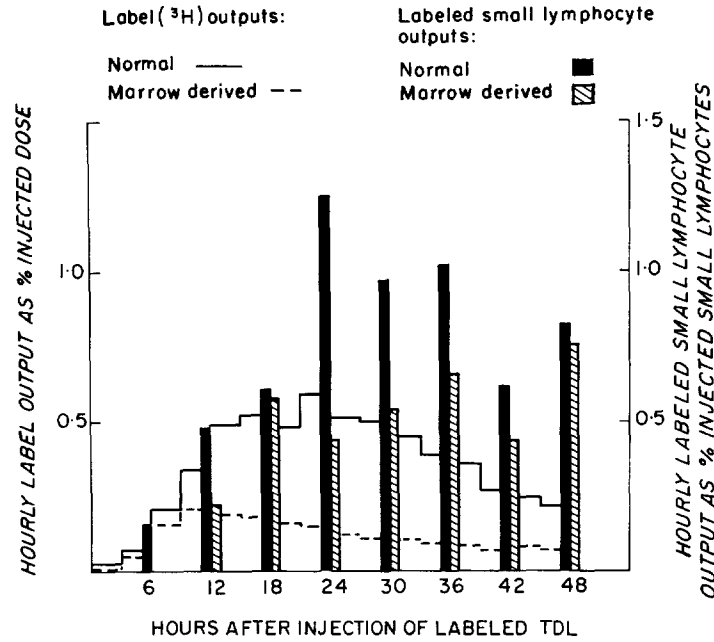


FIG. 4. Recirculation of normal and marrow-derived small lymphocytes in normal recipients. Normal and marrow-derived thoracic duct cells were labeled with uridine- ^3H (one expt each) and transferred intravenously to normal recipients, bearing thoracic duct fistulae. Cell-associated radioactivity in recipient lymph (label output) expressed as in Fig. 3. The output of labeled small lymphocytes was estimated on radioautographs prepared from recipient lymph every 6 hr, and is shown in the superimposed line histograms. Small lymphocytes made up 97% of injected normal thoracic duct cells and 80% of injected marrow-derived cells.

taneously with thymidine- ^{14}C and uridine- ^3H , were transferred to normal recipients bearing established thoracic duct fistulae. The mean total recovery of ^{14}C label was only 1.5% of the injected dose (Fig. 3), implying that little large lymphocyte-associated uridine label could recirculate into the lymph. The tempo of recovery of thymidine- ^{14}C and its magnitude relative to uridine- ^3H recovery (Fig. 3) suggested that some but not all of the uridine label recovered after transfusion of marrow-derived lymphocytes could be attributed to the minority of heavily labeled recirculating large lymphocytes. After an early maximum under the 12 hr uridine mode, the recovery of thymidine- ^{14}C fell rapidly to low levels, while the recovery of uridine- ^3H remained relatively

high. This suggested that the recirculation of large lymphocytes or their progeny could account for the early mode of uridine recovery but that the level of uridine recovery thereafter was due to the recirculation of another population of cells. This second population of recirculating cells was shown by radioautography to be small lymphocytes.

Recirculation of Marrow-Derived Small Lymphocytes Shown by Radioautography.—Thoracic duct lymphocytes obtained from B rats reconstituted with 10^7 lymphocyte-depleted marrow cells 68 days before cannulation, and from

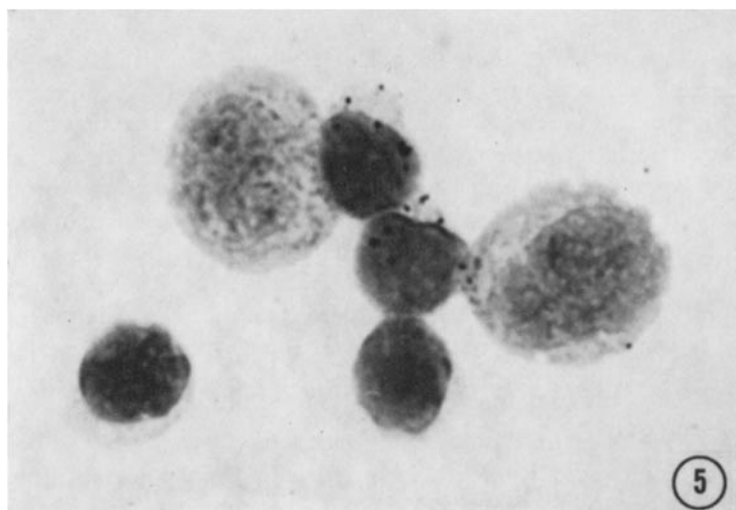


FIG. 5. Two marrow-derived small lymphocytes labeled with uridine- ^3H in the thoracic duct lymph of a normal recipient 48 hr after transfer. Extremely light labeling is characteristic of marrow-derived small lymphocytes (14 day exposure). $\times 2000$.

normal donors, were labeled with uridine- ^3H and transfused into normal recipients bearing thoracic duct fistulae. To minimize reutilization of label in vivo, recipients were infused intravenously with uridine- ^1H at a dose rate of $4 \mu\text{M}/\text{hr}$ from 1 hr before cell transfer until the end of the experiment. Lymph was collected from the thoracic duct and prepared for scintillation counting in 3-hr pools. The number of cells in each sixth hourly fraction was counted and labeled small and large lymphocytes in these fractions were recorded in smears prepared for radioautography. The total numbers of labeled small lymphocytes recovered in each sixth hourly fraction are recorded in Fig. 4 as a percentage of the injected dose of small lymphocytes. These data, superimposed on the simultaneous recovery of radioactivity show that the recirculation of marrow-derived small lymphocytes from blood to lymph is a major traffic, comparable in magnitude to the recirculation of small lympho-

cytes taken from the thoracic duct of a normal donor, but different in tempo. The first appearance of marrow-derived small lymphocytes in the thoracic duct was delayed relative to small lymphocytes from normal donors and there was no well-marked modal recovery time. The total recovery of marrow-derived small lymphocytes calculated from the 6-hr estimates over a 48 hr period was 19.5% against 33.0% for small lymphocytes from normal donors. For the reasons given in Materials and Methods, these values will tend to be underestimates and since marrow-derived small lymphocytes were very lightly labeled (Fig. 5), the underestimation will particularly affect the calculated recovery of these cells.

Fig. 4 shows that after 18 hr the recirculation of labeled small lymphocytes from normal donors progressively exceeds that predicted by the recovery of radioactivity. This is probably due partly to loss of radioactivity from small lymphocytes, which has been shown to amount to about 50% over a 24 hr period in vivo (unpublished observations). In the case of marrow-derived lymphocytes, the deficiency of recovered radioactivity relative to recovered labeled small lymphocytes is much greater, as expected from the poor recirculation of the heavily labeled large lymphocytes (Fig. 3). The radioautographs of lymph from recipients of labeled cells also confirmed the early recirculation of large lymphocytes. Of a total of 24 labeled large lymphocytes recorded, 21 were in lymph collected within 24 hr of cell transfer.

DISCUSSION

This paper describes a method for preparing pure populations of marrow-derived lymphocytes in the rat. These cells were obtained from the thoracic duct in adult thymectomized, lethally irradiated, bone marrow-reconstituted rats between 1 and 3 months after marrow reconstitution and were indistinguishable in the light microscope from the large and small lymphocytes in normal rat thoracic duct lymph. Administration of tritiated thymidine to the donors of such lymphocytes showed that the majority of marrow-derived small lymphocytes had a life-span longer than 7 days. Intravenous transfusion of marrow-derived lymphocytes labeled with tritiated uridine into normal recipients showed that the small lymphocytes in the inoculum recirculated from blood to lymph, but with a tempo which distinguished this population from the majority of small lymphocytes from normal donors.

Proof that the population studied represented peripheral marrow-derived lymphocytes rested on the observation that when alloantigenically labeled bone marrow was transferred to irradiated recipients, their thoracic duct lymphocytes expressed alloantigens unique to the marrow donor. Since the 12 hr lymphocyte output of thymectomized, irradiated recipients was not correlated with the number of recirculating lymphocytes contaminating the marrow inoculum,² and was in some cases considerably greater than the total marrow inoculum, it was argued that the thoracic duct lymphocytes present

were generated by mitosis from precursors in the marrow that were probably not themselves recirculating lymphocytes. In the last analysis the identity of the marrow-derived thoracic duct small lymphocyte population is shown by the properties of this population which distinguish it from thoracic duct lymphocyte populations from normal rats. Outstanding among these is the failure of marrow-derived lymphocytes from the thoracic duct to reverse the unresponsiveness to sheep red blood cells of heavily irradiated recipients except in the presence of thymus-derived cells¹ compared with the full competence of normal lymphocyte populations in this response (7, 20). On the grounds of proven marrow origin, thymus-independent maturation and immunological properties it is appropriate to term the thoracic duct small lymphocytes of B rats, "B lymphocytes" (21).

The relevance of the physiological findings described in this paper to the properties of B lymphocytes in normal rats must be considered. Conceivably, a long life-span and the ability to recirculate were abnormal properties acquired by marrow-derived lymphocytes in response to a deficit of thymus-derived lymphocytes. It has, however, recently been shown that the normal rat thoracic duct carries a subpopulation of small lymphocytes which are long-lived judged by their labeling with tritiated thymidine, and when carrying this label can be shown to recirculate from blood to lymph in splenectomized recipients with a modal recovery time of 26–28 hr (13). It has been suggested that these cells, which are selectively enriched by chronic thoracic duct drainage, are the normal representatives of the marrow-derived lymphocytes described in this paper (13). Two recent studies in mice also suggest that some marrow-derived lymphocytes in normal blood and lymphoid tissues may be long-lived (22, 23).

Further reasons for believing that the B lymphocytes described in this paper represent a pure preparation of a population also present in thoracic duct lymph from normal rats, based on a study of uridine uptake and characteristic distribution in lymphoid tissues, are presented in the next paper (18).

It may be expected that in the presence of specific antigen responding subpopulations of marrow-derived lymphocytes will divide repeatedly. Possibly, therefore, the immediate precursors of specific antibody-forming cells will prove to be short-lived cells. The present findings refer specifically to the majority of marrow-derived small lymphocytes in the circulation and should not be generalized to all marrow-derived lymphocytes in all circumstances.

The presence of a long-lived population of marrow-derived small lymphocytes in the thoracic duct virtually guaranteed that this population also normally recirculated from blood to lymph. Low uridine uptake by marrow-derived small lymphocytes, in the presence of a large number of large lymphocytes labeling heavily with uridine, gave a misleading result when recirculation was monitored by scintillation counting alone, but by counting labeled recirculating cells in radioautographs it was possible to arrive at a more accu-

rate figure for the rate of recirculation of marrow-derived small lymphocytes. These data (Fig. 4) show that the recirculation of marrow-derived small lymphocytes is a large scale traffic, with different kinetics from the recirculation of thymus-derived small lymphocytes. The next paper (18) shows histologically that the routes of recirculation of the two populations through lymph nodes and spleen are also distinct.

SUMMARY

These experiments describe the preparation of pure marrow-derived lymphocyte suspensions from the thoracic duct of thymectomized, irradiated rats reconstituted with bone marrow cells. The majority of marrow-derived cells were small lymphocytes morphologically indistinguishable from small lymphocytes in thoracic duct lymph of normal donors.

Marrow-derived small lymphocytes (B lymphocytes) were a predominantly long-lived population; the frequency of short-lived B lymphocytes in the thoracic duct was not significantly higher than the frequency of short-lived small lymphocytes in normal lymph.

B lymphocytes transferred to normal recipients recirculated from blood to lymph. The first appearance of intravenously injected B lymphocytes in the thoracic duct was delayed relative to lymphocytes from normal donors and there was no clear cut modal recirculation time. Nevertheless their recirculation over a 48 hr period after transfusion was of the same order of magnitude as that of lymphocytes from normal donors.

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