Reentry of T Cells to the Adult Thymus Is Restricted to Activated T Cells

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Summary

To seek information on the capacity of mature T cells to migrate to the thymus, mice were injected with Thy-1-marked populations enriched for resting T cells or T blast cells; localization of the donor cells in the host thymus was assessed by staining cryostat sections of thymus and by FACS® analysis of cell suspensions. With injection of purified resting T cells, thymic homing was extremely limited, even with injection of large doses of cells. By contrast, in vivo generated T blast cells migrated to the thymus in substantial numbers. Thymic homing by T blasts was >50-fold more efficient than with resting T cells. Blast cells localized largely in the medulla and remained in the thymus for at least 1 mo post-transfer. Interestingly, localization of T blasts in the thymus was 10-fold higher in irradiated hosts than normal hosts. Thymic homing was especially prominent in mice injected with T blasts incubated in vitro with the DNA precursor, ^{125}I-5-iodo-2'deoxyuridine (^{125}IDUR); with transfer of ^{125}IDUR-labeled blasts to irradiated hosts, up to 5% of the injected counts localized in the host thymus. These data suggest that thymic homing by T blasts might be largely restricted to cells in S phase. The physiological significance of blast cell entry to the thymus is unclear. The possibility that these cells participate in intrathymic tolerance induction is discussed.

In addition to immature cells, the thymus contains small numbers (~10%) of mature T cells (1, 2). These cells are situated in the medulla. Since the T cells exiting from the thymus have a mature phenotype (1), the cells of the medulla are generally viewed as immunocompetent virgin T cells in the process of being exported to the secondary lymphoid tissues. This notion rests on the assumption that lymphocyte traffic from the thymus is strictly unidirectional. However, there are a number of reports that T cell lines or mature lymphocytes harvested from the secondary lymphoid organs are able to localize in the thymus in appreciable numbers after intravenous injection (3–6). These cells localize largely in the medulla.

The physiological significance of "back migration" of T cells into the thymus is unknown. One possibility is that T cells entering the thymus from the periphery carry unique self antigens not represented in the thymus. Presentation of these antigens in the thymus could be important for self tolerance induction. A precedent for this idea has come from the finding that intravenous injection of Mls^a T cells into Mls^b neonatal mice causes intrathymic tolerance to Mls^a antigens (7), implying that the injected T cells enter the thymus and tolerize newly formed host T cells.

The observation that some T cells have the capacity to reenter the thymus from the periphery questions the assumption that mature thymocytes are virgin cells recently derived from the cortex. Indeed, one has to consider the possibility that many of the functional T cells found in thymocyte suspensions represent thymic immigrants. This could explain why transgenic mice expressing I-E alloantigens selectively in the pancreas can show tolerance not only in spleen and lymph nodes (LN), but also in the thymus (8).

The main aim of the experiments in this paper was to examine the extent of mature T cell migration to the thymus and the types of T cells involved. The results show that typical mature resting T cells from LN have virtually no capacity to migrate to the thymus of adult mice. By contrast, activated T cells readily enter the thymus; these cells lodge in the medulla and remain there for prolonged periods.

Materials and Methods

Mice. C57BL/6 (B6), B6.PL Thy-1^a (B6.PL), B6.C-H-2^{bm1} (bm1), B6.C-H-2^{bm12} (bm12), CBA/Ca, and F_1 hybrids between these strains were raised and maintained at the Research Institute of Scripps Clinic.

Irradiation. Mice were exposed to irradiation from a ^{137}Cs source (80 rad/min) delivered by a Gammacell 40 irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada).

mAbs. The following mAbs were used: biotinylated anti-Thy-1.2 (Jlj, rat IgG) (9); biotinylated anti-Thy-1.1 (19E12, mouse IgG) (10); anti–heat-stable antigen (Jlld, rat IgM, culture supernatant)

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(9); anti-CD4 (GK1.5, rat IgG2b, ascites) (11); and anti-CD8 (3.168.8, rat IgM, ascites) (12).

Purification of Resting T Cells. LN cells prepared from pooled cervical, axillary, mesenteric, and inguinal nodes were passed through nylon wool (NW)[1] columns and separated on Percoll gradients to prepare small (high density) resting T cells (13).

Generation of T Blasts In Vivo. Purified T cells or T cell subsets prepared from parental strain LN were transferred intravenously to F_1 hybrid mice exposed to 900–1,000 rad 2–4 h before. To obtain circulating blast cells, thoracic duct cannulas were inserted at 3–4 d post-transfer (14, 15); blasts cells were collected over 8–16 h.

Staining of Cryostat Sections. Freshly removed thymuses were frozen in liquid nitrogen and 5–6-μm sections were cut in a cryostat. The sections were dried overnight, fixed in acetone for 2 min, and incubated with optimal concentrations of biotinylated anti-Thy-1.2 or anti-Thy-1.1 mAb for 1 h. After washing, horseradish peroxidase–conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA) was added for additional 30 min; after further washing, sections were incubated with the substrate 3-amino-9-ethylcarbazole (0.1 mg/ml in 0.05 M NaOAc, pH 5.2) with 0.01% H_2O_2 for 20 min. The sections were then washed again, counterstained with Mayer's Hematoxylin (Sigma Chemical Co., St. Louis, MO), and photographed.

FACS® Analysis. Thymocytes, PBL, LN, and spleen cells were stained for the presence of the injected donor cells by incubating with biotinylated anti-Thy-1.2 or anti-Thy-1.2 mAbs for 20 min on ice. Cells were washed and incubated with FITC-conjugated streptavidin (Zymed, San Francisco, CA) and propidium iodide (2 μg/ml) for 20 min. Viable cells were analyzed by FACS IV® or FACSCAN® (Becton Dickinson & Co., Mountain View, CA). Thymocytes from nonirradiated recipients were usually treated with Jlld + C and enriched for viable cells by low ionic salt buffer column (13) before staining.

Labeling with ^{125}I-5-iodo-2' Deoxyuridine (^{125}IDUR). As described elsewhere (14), T blast cells or bone marrow cells were incubated with ^{125}IDUR (sp act, 2,000 Ci/mmol) (The Radiochemical Centre, Amersham, England) at 1 μCi/ml/2 × 10^7 cells for 1 h at 37°C in RPMI 1640 supplemented with 10% FCS. After washing four times, labeled cells were injected intravenously into normal mice or mice exposed to 1,000 rad 3 h before; the injected cells (~10^7/mouse) contained ~40,000 cpm. Groups of the recipients were killed at intervals to remove organs for radioactive counting. Radioactivity levels were counted using an automatic gamma counter (4/600; Micromedic Systems, San Clemente, CA). The percent localization of the injected counts was calculated with reference to an aliquot of the injected cells.

Results

Experimental Design. In most experiments, the approach used was to inject young (6–10 wk) mice with Thy-1-marked T cells intravenously and then search for donor cells in the host thymus and other organs using FACS® analysis. B6 (H-2^b, Thy-1.2) T cells were transferred to B6.PL (H-2^b, Thy-1.1) mice, or vice versa. Preliminary experiments in which B6 and B6.PL thymocytes were mixed together in defined proportions and then stained for Thy-1.2 vs. Thy-1.1 showed that cell ratios of 1:1,000 were easily detected.

Homing of Resting T Cells. When FACS® analysis was performed on unseparated thymocytes, intravenous injection of even large doses of normal LN T cells led to no discernible localization of the donor T cells in the host thymus (<0.01%) (data not shown). In subsequent experiments, thymocyte suspensions were selectively depleted of immature cells (~95% of thymocytes) by treating the suspensions with Jlld mAb + C before staining and FACS® analysis (9); this treatment spares fully mature T cells (including T blast cells) and thus enhances the detection of immigrant T cells by ~20-fold.

When B6 mice were injected with a dose of 4 × 10^7 unseparated B6.PL LN cells taken from normal young donors, homing of the donor T cells to the host thymus was virtually undetectable (Table 1, Exp. 1). Thus, <0.01% of Jlld$^-$ thymocytes expressed the Thy-1.1 maker of the donor. This applied at days 1, 5, and 10 post-transfer. In contrast to the thymus, the donor T cells were easily detectable in spleen and LN (5–10%). With the reciprocal B6 → B6.PL combination, a shortage of mice necessitated the use of older (5–6 mo) donor mice in initial experiments. Two experiments with these donors led to significant thymic homing, i.e., ~0.2% of Jlld$^-$ cells (data not shown). In subsequent experiments, young (<2 mo) donors were used and the T cells were passed through NW columns before injection. As shown in Table 1 and Fig. 1 *c*, thymic homing in B6.PL hosts given 4 × 10^7 NW-passed B6 LN cells was extremely low, i.e., ≤0.04% of Jlld$^-$ cells; thymic homing reached only 0.08% in a mouse given 2 × 10^8 NW-passed cells. Cryostat sections of thymus revealed occasional donor-derived T cells in the medulla (Fig. 1 *d*), but these cells were very rare. When the injected T cells were NW passed and then separated on Percoll gradients to prepare purified small resting T cells, thymus homing was undetectable (<0.01%) (Table 1, Exp. 2).

To examine whether irradiation increases the permeability of the thymus to peripheral T cells, some of the host mice were exposed to 1,000 rad a few hours before T cell transfer. This dose of irradiation destroys ~98% of thymocytes within 24 h. Since the surviving cells are nearly all mature T cells, the thymocyte suspensions were not subjected to Jlld + C treatment. As shown in Table 1, prior irradiation of the host failed to augment T cell homing to the thymus. Similar findings applied to hosts pretreated with hydrocortisone (Table 1); like irradiation, injection of hydrocortisone causes severe thymic atrophy.

Homing of T Blast Cells. The above data imply that resting T cells have little if any capacity to home to the thymus. To examine thymic homing by activated T cells, Con A blasts were used in initial experiments. These cells homed very poorly, even to spleen, and were not studied further. In subsequent experiments, in vivo blasts were used. T blasts were generated by transferring Thy-1-marked parental strain T cells to irradiated (900 rad) H-2-different F_1 hybrid mice. Under these conditions, the donor T cells mount a strong proliferative response to the host alloantigens, especially in the spleen, and then enter the circulation in large numbers as blast cells (14, 15). Two approaches were used to measure thymic homing by the circulating blast cells.

[1] *Abbreviations used in this paper:* ^{125}IDUR, ^{125}I-5-iodo-2' deoxyuridine; NW, nylon wool.

Table 1. *Failure of Resting T Cells to Migrate to the Thymus of Normal and Irradiated Hosts*

Exp.	Cells injected	Donor → host	Time after adoptive transfer	Mean percent of donor cells in: Thymus (Jlld⁻)	Spleen	LN
			d			
1	4×10^7 LN	B6.PL → B6	1	< 0.01 (10)*	14.9 (10)	4.9 (10)
			3	< 0.01 (8)	9.4 (8)	4.5 (8)
			10	< 0.01 (8)	10.1 (8)	5.7 (8)
		B6.PL → B6 (1,000 rad)	1	< 0.01 (8)‡	40.1 (8)	19.2 (8)
		B6.PL → B6 (CRT)§	1	< 0.01 (4)	12.7 (4)	2.6 (4)
2	4×10^7 LN T (NW)	B6 → B6.PL	1	0.02 (2)	10.1 (2)	3.8 (2)
	4×10^7 LN T (NW, Percoll)		1	< 0.01 (2)	8.2 (2)	3.5 (2)
3	4×10^7 LN T (NW)	B6 → B6.PL	1	0.03 (2)	4.0 (2)	5.0 (2)
4	2×10^8 LN T (NW)	B6 → B6.PL	1	0.08 (1)	15.5 (1)	ND
5	4×10^7 LN T (NW)	B6 → B6.PL	1	0.02 (2)	4.0 (2)	6.0 (2)
			5	0.08 (2)	2.8 (2)	6.1 (2)
		B6 → B6.PL (1,000 rad)	1	0.03 (2)	15.1 (2)	50.8 (2)

Young normal mice or mice exposed to 1,000 rad 3 h before were injected intravenously with unseparated LN cells or LN cells passed through NW columns; after NW, some cell suspensions were separated on Percoll gradients to prepare small dense T cells (see Materials and Methods). The host mice were killed at 1–10 d post-transfer to prepare cell suspensions from thymus. For unirradiated hosts, thymocyte suspensions were treated with Jlld mAb + C before staining to enrich for mature T cells; cell yields from Jlld mAb + C-treated thymus were ~ 5%. Cell suspensions were stained for Thy-1.1 or Thy-1.2 and analyzed on a FACS® (see Materials and Methods). Nonspecific staining was checked in each experiment, e.g., by staining normal and Jlld⁻ thymocytes, LN, and spleen cells from an age-matched uninjected mouse. The level of nonspecific staining for Jlld⁻ thymocytes was very low (≤0.03, see Fig. 1 *a*). The value obtained for nonspecific staining has been subtracted from the data shown.

* Mean number of cells expressing donor Thy-1.1 marker; the number of mice tested is shown in parenthesis.

‡ Thymocyte suspensions from irradiated mice were not treated with Jlld mAb + C before staining.

§ Cortisone-resistant thymocytes. Mice received 2.5 mg hydrocortisone intraperitoneally 1 d before; the thymus showed severe atrophy, and CRT were not treated with Jlld + C.

Blast Cell Homing In Situ. The first approach was to allow the blast cells to enter the endogenous thymus of the irradiated host. The experiments shown in Table 2 used donor/host combinations differing selectively at H-2 class I or class II loci. By transferring CD4⁺ LN T cells to class II–different recipients (e.g., B6.PL CD4⁺ → [B6 × bm12]F_1) and CD8⁺ cells to class I–different recipients (e.g., B6.PL CD8⁺ → [B6 × bm1]F_1), it was possible to compare the homing of CD4⁺ vs. CD8⁺ blasts. Host thymuses were removed at days 4–6 post-transfer and stained for Thy-1.1 vs. Thy-1.2 expression. Unseparated thymocytes were used for staining: thymuses were small (a consequence of irradiation) and contained $<10^6$ viable cells.

As shown in Table 2 and Fig. 1, *e* and *f*, homing of donor blasts to the host thymus was considerable, both for CD4⁺ and CD8⁺ blasts. The extent of thymic homing was roughly proportional to the numbers of T cells initially injected and was higher on day 6 (the latest time examined) than on days 4 or 5. With class II–different combinations, up to 25% of total thymocytes were of donor origin (Fig. 1 *e*). With class I–different combinations, the proportion of donor-derived cells in the thymus reached 50–70%. Tissue sections revealed dense accumulations of donor T cells spread throughout the thymus (Fig. 1 *f*); because of irradiation damage, the thymus showed marked atrophy with no obvious cortico-medullary demarcation. In a similar system, Fukushi et al. (16) recently reported that large numbers of $V_\beta 6^+$ blast cells enter the thymus of Mls^a-different irradiated mice with GVHD.

The above data refer to irradiated hosts. When the hosts were not irradiated, donor-derived blasts accounted for ~2% of Jlld⁻ thymocytes (Table 2). Total yields of donor T cells in the thymus were three- to fourfold higher in irradiated hosts than in unirradiated hosts. Thus, with transfer of 10^7 CD4⁺ cells to class II–different irradiated mice, the total yield of donor-derived T cells in the thymus on day 6 amounted to 1.5×10^5 cells/mouse for irradiated hosts and 0.5×10^5 cells/mouse for normal hosts.

Blast Cell Homing on Adoptive Transfer. Because of (*a*) the H-2 disparity between the donor T cells and the host thymus and (*b*) the ongoing graft-vs.-host reaction, the thymic homing of T blasts seen in the above experiments is of dubious physi-

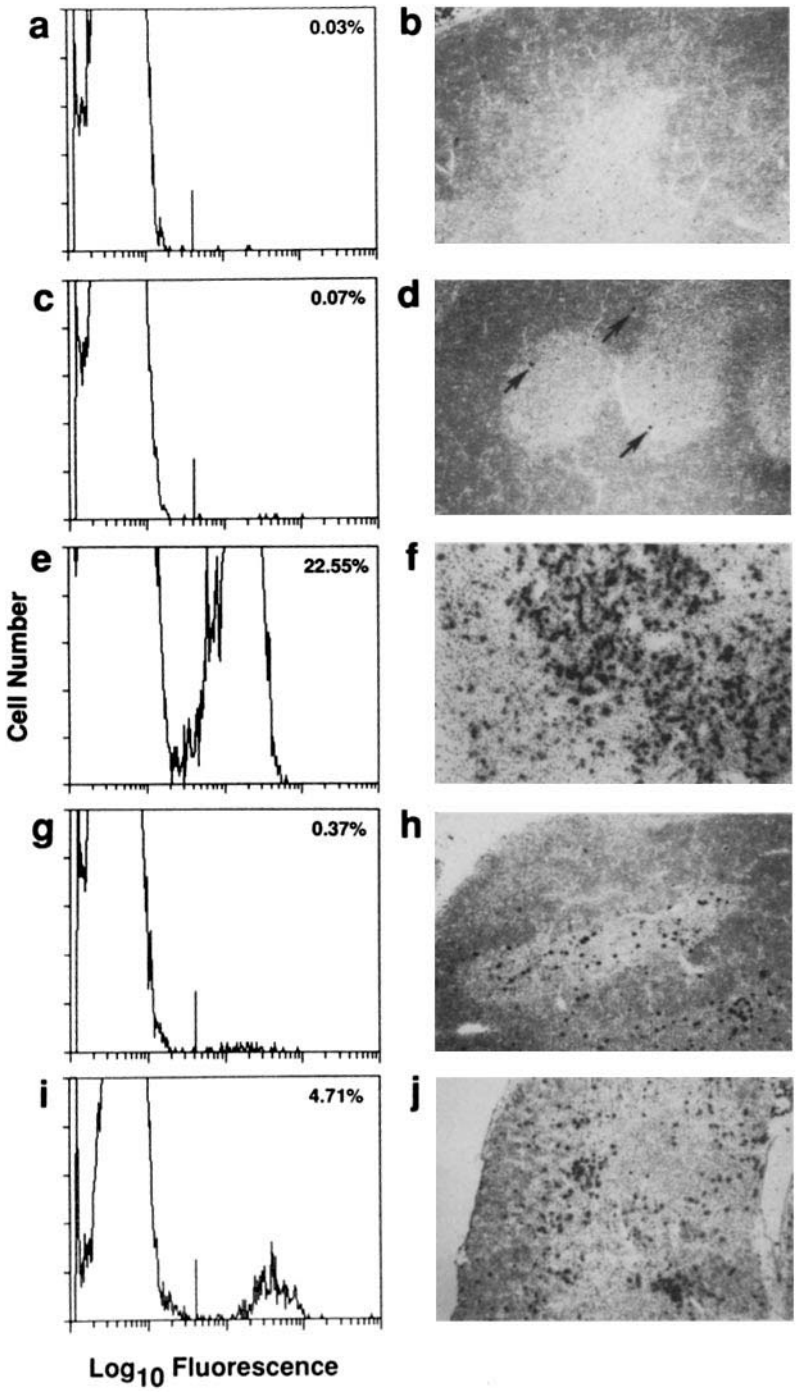

Figure 1. Detection of Thy-1-marked donor T cells in thymus of hosts injected with T cells under various experimental conditions. Thymuses from Thy-1.1 (B6.PL) host mice were stained for Thy-1.2 expression after intravenous injection of Thy-1.2$^+$ T cells. Donor Thy-1.2$^+$ T cells were detected by FACS® analysis of thymocyte suspensions or by staining cryostat sections of thymus; except for irradiated hosts, Jlld$^-$ thymocyte suspensions were used for FACS® analysis. (*a* and *b*) Thymus of uninjected B6.PL mouse (negative control). (*c* and *d*) Thymus of B6.PL mouse injected 1 d before with 4 × 10^7 NW-passed B6 LN cells. Thy-1.2$^+$ cells are very rare in cell suspensions but can be seen as occasional cells (*arrows*) in the medulla in tissue sections. (*e* and *f*) Thymus of irradiated (1,000 rad) B6.PL mouse injected 5 d before with 10^7 class II–different bm12 CD4$^+$ cells. Thy-1.2$^+$ cells are easily detectable in cell suspensions and are scattered throughout the thymus in tissue sections; because of irradiation, the cortex and medulla are poorly demarcated. (*g* and *h*) Thymus of normal unirradiated B6.PL mouse injected 1 d before with 4 × 10^7 in vivo generated B6 blast cells. Thy-1.2$^+$ cells are detectable in cell suspensions and are easily visualized in the medulla in tissue sections. (*i* and *j*) Thymus of irradiated (1,000 rad) B6.PL mouse injected 1 d before with 4 × 10^7 in vivo generated B6 T blast cells. Thy-1.2$^+$ cells are common in cell suspensions and are scattered throughout the thymus in tissue sections.

ological significance. The experiments in Table 3 show homing of T blasts after adoptive transfer to syngeneic hosts. Blasts generated in parent → F_1 combinations were collected from thoracic duct lymph of the irradiated hosts at 4–5 d post-transfer. At this stage, the vast majority of the lymph-borne cells are typical blasts cells (14, 15). These cells exhibit the phenotype of activated T cells and show excellent viability (>99%). T blasts were generated in hosts expressing either combined class I plus class II disparities (B6 or B6.PL → [B6 × CBA/Ca]F_1) or class II antigens alone (B6 or B6.PL → [B6 × bm12]F_1). To study homing, B6 blasts were transferred to B6.PL hosts, or vice versa.

As shown in Table 3 and Fig. 1, *g–j*, homing of T blasts to H-2-compatible thymuses was low but clearly significant. With normal unirradiated mice as hosts, injection of 4 × 10^7 blast cells yielded ~0.4% donor cells in the Jlld$^-$ component of host thymocytes (Fig. 1 *g*); donor cells were easily detectable in tissue sections and were largely restricted to the

Table 2. *Distribution of Donor T Cells in Irradiated Hosts Expressing Class I or II H-2 Differences: Widescale Entry of Blast Cells into the Host Thymus*

Exp.	Donor → host	H-2 barrier	No. of T cells injected	Time after adoptive transfer	Mean percent of donor cells in:		
					Thymus	LN	PBL
				d			
1	bm12 $CD4^+$ → 900 rad B6.PL	Class II	10^7	4	12.8	ND	75.0
				5	19.1	ND	81.0
2	B6.PL $CD8^+$ → 900 rad (B6 × bm1)F_1	Class I	10^7	5	31.2	92.0	ND
				6	51.3	98.0	ND
3	B6.PL $CD4^+$ → 1,000 rad (B6 × bm12)F_1	Class II	10^5	6	1.4	44.0	ND
			10^6	6	21.6	73.0	ND
			10^7	6	26.1	71.0	ND
4	B6.PL $CD8^+$ → 1,000 rad (B6 × bm1)F_1	Class I	10^5	6	0.2	19.0	ND
			10^6	6	12.9	84.7	ND
			10^7	6	73.4	90.0	ND
5	B6.PL $CD4^+$ → (B6 × bm12)F_1 (host not irradiated)	Class II	10^7	6	1.8*	2.7	ND

Various doses of purified $CD4^+$ or $CD8^+$ cells prepared from LN by mAb + C treatment were injected intravenously into H-2-different, Thy-1-different hosts exposed to irradiation 3 h before. At 4–6 d after transfer, suspensions of thymocytes, LN, and/or PBL were stained and analyzed for donor Thy-1 expression; cell yields from irradiated thymuses were very low, i.e., $<10^6$ cells/mouse. The data show the mean percent of donor cells from two mice/group. Nonspecific staining was determined and subtracted for each experiment as described in Table 1.
* Percent donor cells in $Jlld^-$ fraction of thymocytes.

medulla (Fig. 1 *h*). This applied irrespective of whether the blasts were predominantly $CD4^+$ (generated in class II–different hosts) or a 2:3 mixture of $CD4^+$ and $CD8^+$ cells (generated in class I + II–different hosts). The proportion of donor T cells in host spleen and LN ranged from 3 to 6%. Interestingly, the frequency of donor cells in the host thymus remained relatively constant for prolonged periods. Thus, whereas the percent of donor cells in the spleen declined substantially after several days, the percent of donor cells in the thymus remained largely unchanged for at least 28 d post-transfer (Table 3). The above data apply to the percent of donor T cells detected in $Jlld^-$ thymocytes. When total yields of these cells were taken into consideration, $Jlld^-$ thymocyte suspensions contained ~20,000 donor-derived cells, i.e., ~0.05% (1:2,000) of the injected dose of 4×10^7 blasts.

Thymic homing of blast cells was substantially higher in the thymus of irradiated hosts (hosts exposed to 1,000 rad 3 h before transfer) (Table 3; Fig. 1, *i* and *j*). In this situation, 5–19% of unseparated thymocyte suspensions were donor derived when measured at 1 or 5 d post-transfer. In terms of total cell yields, ~0.5% of the injected blasts reached the thymus of irradiated hosts, i.e., 10-fold higher than in unirradiated mice.

Homing of ^{125}IDUR-labeled Blasts. To examine whether homing of blast cells to the thymus extends to cells in S-phase, thymic homing of ^{125}IDUR-labeled blasts was studied. In vivo generated blast cells were incubated with ^{125}IDUR (a DNA precusor) at 1 μCi/ml for 1 h in vitro, washed thoroughly, and then transferred intravenously to syngeneic normal mice or irradiated (1,000 rad) mice. Groups of the recipients were killed at 1, 3, 6, or 20 h post-transfer. The percent of the injected radioactivity recovered from the whole thymus and spleen of the hosts is shown in Fig. 2. With unirradiated mice as hosts, thymic homing was low (~0.1%) at 1 h post-transfer but increased progressively to reach ~0.5% at 20 h (Fig. 2 *a*). Thymic homing was also low initially in irradiated hosts but then increased dramatically to reach 4.7% by 20 h; essentially similar results were seen in a second experiment (3.3% in the thymus of irradiated hosts at 20 h compared with 0.5% in normal hosts). In contrast to the thymus, blast cell homing to the spleen was only slightly higher in irradiated hosts than normal hosts (Fig. 2 *b*).

As with Thy-1-marked blasts, the above data indicate that thymic homing with ^{125}IDUR-labeled blasts is 10-fold higher in the irradiated thymus than in normal thymuses. The unexpected finding, however, is that the percent of the transferred donor cells localizing in the thymus is far higher

Table 3. *Thymic Homing of T Blasts after Transfer to Normal Syngeneic Hosts vs. Irradiated Hosts*

Exp.	Donor → host	Time after adoptive transfer	Mean percent of donor cells in:		
			Thymus (Jlld⁻)	Spleen	LN
		d			
1	4 × 10^7 B6 T blasts → B6.PL	1	0.26	6.2	ND
2	4 × 10^7 B6 T blasts → B6.PL	1	0.42	3.6	3.2
		3	0.61	3.3	1.8
		14	0.40	0.6	0.7
3	4 × 10^7 B6.PL T blasts → B6	1	0.36	3.7	ND
		14	0.48	1.0	ND
		28	0.57	0.9	ND
4	4 × 10^7 B6 T blasts → B6.PL (1,000 rad)	1	11.3	40.0	ND
5	4 × 10^7 B6 T blasts → B6.PL (1,000 rad)	1	6.1	15.4	ND
6	4 × 10^7 B6.PL T blasts → B6 (1,000 rad)	1	8.0	36.9	ND
		5	10.1	51.1	ND
7	2 × 10^7 B6.PL T blasts → B6 (1,000 rad)	1	19.0	ND	4.3

Doses of 1–2 × 10^7 purified LN T cells from B6 or B6.PL mice were transferred intravenously to (B6 × bm12)F_1 mice (class II difference) or (B6 × CBA/Ca)F_1 mice (class I + II difference) exposed to 900 rad 3 h before. The recipients were cannulated 3 or 4 d later, and thoracic duct cells were collected on ice over intervals of 8–16 h; the vast majority of these cells (80–90%) had features of blast cells. The lymph-borne cells were transferred intravenously to normal or irradiated H-2-compatible Thy-1 different hosts. The data show the mean percent of cells expressing the donor Thy-1 marker recovered from two to four mice/group. The homing properties of blasts enriched for CD4⁺ cells (class II differences) or CD8⁺ cells (combined class I + II differences) were not discernibly different; for simplicity, the hosts in which the blasts were generated are not shown. Nonspecific staining was determined and subtracted from each experiment, as described in Table 1.

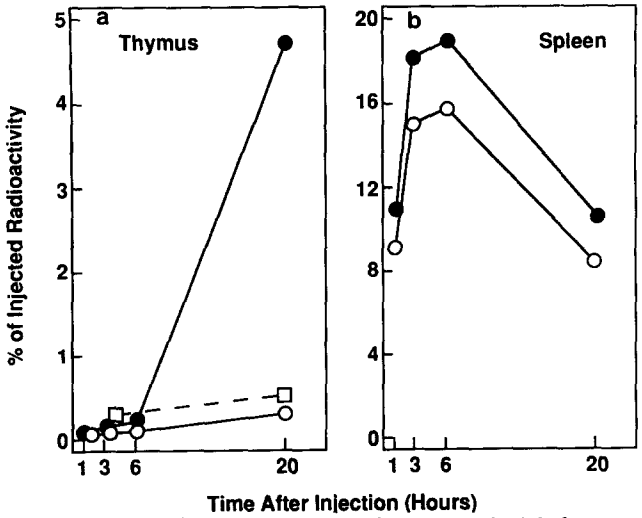

Figure 2. Normal mice or mice exposed to 1,000 rad 3 h before were injected intravenously with syngeneic T blasts labeled in vitro with 1 μCi/ml ^{125}IDUR followed by extensive washing; T blasts were obtained from thoracic duct lymph of irradiated (B6 × bm12)F_1 mice injected 4 d before with 2 × 10^7 B6 LN T cells. Groups of the recipients of labeled T blasts were killed at intervals to remove whole thymuses and spleen for radioactive counting. The data show the mean percent of the injected radioactivity recovered from two mice/group relative to an aliquot of the injected cells. (○) Labeled T blasts transferred to normal hosts; (●) labeled T blasts transferred to irradiated hosts; (□) labeled bone marrow cells transferred to irradiated hosts.

with ^{125}IDUR-labeled blasts (up to 5% in the irradiated thymus) than with unseparated (Thy-1-marked) blast cells (0.5% in the irradiated thymus). To rule out artifact, control experiments were performed with ^{125}IDUR-labeled normal bone marrow cells. Homing of these non-T cells to the thymus of irradiated hosts was quite low, i.e., 0.5% of the injected counts at 20 h post-transfer (Fig. 2 *a*). When ^{125}IDUR-labeled cells were heat killed (20 min incubation at 60°C) before injection, the thymus of irradiated mice contained <0.1% of the injected counts at 20 h (data not shown).

Discussion

The data in this paper suggest that homing of mature T cells to the thymus of young adult mice is large and perhaps exclusively restricted to activated T cells. The observation that thymic homing was very low with normal LN cells and undetectable when LN cells were NW/Percoll gradient separated to prepare small cells implies that mature resting T cells have virtually no capacity to migrate to the thymus. In previous studies, Michie et al. (5) reported finding small numbers of donor T cells in sections of thymus from mice injected with unfractionated LN cells. In view of the present findings, it would seem likely that these few cells were activated T cells.

The notion that circulating resting T cells are excluded from entering the thymus is in accord with the evidence that

typical small lymphocytes remain within the confines of the recirculating lymphocyte pool (2, 17). Except for the spleen, recirculating lymphocytes stay in the circulation (blood or lymph) unless the cells make contact with high endothelial venules (HEV). In healthy young animals, the distribution of HEV is largely restricted to LN and Peyer's patches. In vitro studies have shown that lymphocytes overlaid on frozen sections of LN adhere strongly to HEV via their homing receptors (18, 19). Such binding is very limited on sections prepared from normal thymus (E. Butcher, personal communication). Interestingly the thymus is reported to contain "post-capillary venules" filled with lymphocytes (20). The failure of peripheral T cells to adhere to these venules in vitro implies that these vessels are largely concerned with exporting T cells from the thymus rather than with T cell import.

In contrast to resting T cells, populations of T blast cells activated to H-2 alloantigens in vivo showed a definite tendency to localize in the thymus, both in situ and on adoptive transfer to syngeneic hosts. Thymic homing of T blast cells was easily detectable in tissue sections and was largely restricted to the medulla, i.e., the main site of mature T cells. Why T blast cells are so much more efficient than resting T cells at homing to the thymus is unclear. The simplest idea is that activation of T cells results in the expression of certain homing receptors, which enables the cells to make contact with complementary molecules on thymic blood vessels. This notion raises the issue of whether thymic homing is restricted to a certain stage of cell cycle. Here, it is worth pointing out that the in vivo blasts used in this study show considerable heterogeneity in cell size and surface markers. Moreover, despite the fact that continuous in vivo infusion of [^{3}H]TdR for 3 d labels >95% of the blast population (15), incubating the blasts for 1 h in vitro labels only 10–15% of the cells (14). The proportion of the cells in S phase is thus quite low. In this respect, it is notable that thymic homing appeared to be much higher with ^{125}IDUR-labeled blasts than with the unseparated blast population. It is conceivable, therefore, that thymic homing of blast cells is largely or entirely restricted to cells in S phase. Further work will be needed to assess this possibility.

It is of interest that blast cell homing was much higher in the thymus of irradiated hosts than in normal hosts. Indeed, with injection of ^{125}IDUR-labeled T blasts, the thymus of irradiated hosts contained up to 5% of the injected counts at 1 d post-transfer. This is a dramatic recovery considering that only 8% of the injected counts were found in the spleen. How irradiation potentiates thymic homing of T blasts is obscure. The possibility that irradiation causes nonspecific damage to thymic blood vessels seems unlikely because irradiation failed to enhance thymic homing by resting T cells. An alternative possibility is that T blasts are drawn to the thymus by cytokines or other mediators released from the massive numbers of dead and dying thymocytes in the irradiated thymus. This notion is difficult to assess experimentally.

The long-term survival of T blasts in the thymus deserves comment. In the extrathymic environment, T blasts home initially to the spleen, liver, and gut (14); most of these cells then disappear as a consequence of a cell death in situ and excretion through the gut wall. The present finding that T blasts remain in the thymus for at least 4 wk with no decline in their numbers is thus highly surprising. Nevertheless, the data are in accord with reports that T cells stimulated with myelin basic protein (3, 4) or minor histocompatibility antigens (21) can enter the thymus and remain there for at least 2 mo post-transfer. Since the cells recovered from the thymus in these latter studies expressed effector activity only after reactivation with antigen in vitro, blast cells entering the thymus presumably revert rapidly to resting cells.

The physiological significance of blast cell migration to the thymus is unclear. As discussed earlier (see Introduction), passive transfer of self antigens into the thymus might serve to promote self tolerance induction. It is notable that autoreactive T cells can return to the thymus after activation in the periphery (3, 4). Such homing could be a device to inhibit the further production of T cells with similar self specificity; the blasts might carry MHC/self antigen complexes (see references 22 and 23) into the thymus and display these complexes to newly formed T cells in tolerogenic form. Alternatively, idiotypic determinants on immigrant blasts cells might stimulate antiidiotypic T cells with autoregulatory function (24, 25). On a more pragmatic note, it is important to know whether immigrant T cells make a significant contribution to the immunocompetence of thymocytes. As discussed earlier (see Introduction), one explanation for the paradoxical intrathymic tolerance seen in certain I-E/insulin promoter transgenic mice (8) is that the functional T cells in the thymus are almost entirely of exogenous origin. This notion rests on the assumption that entry of mature T cells into the thymus is substantial. In the present studies, however, immigrant T cells accounted for only a small fraction of mature thymocytes (<1%) in normal hosts, even when blast cells were transferred in relatively large doses. For this reason, the possibility that most of the functional T cells in the thymus represent thymic immigrants seems rather unlikely.

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