

RARE PERIPHERAL T CELLS MIGRATE TO
AND PERSIST IN NORMAL MOUSE THYMUS

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Thymocyte precursors from bone marrow home to thymus, where they undergo maturational events leading to the formation of functional T cells (1). These T cells are released from thymus to recirculate through peripheral lymphoid organs, such as spleen and lymph node (LN) (2). Although this traffic of mature T cells from thymus to the periphery is generally considered to be unidirectional, abnormal lymphoid cells, such as leukemia cells (3) or cells from in vitro antigen-activated T cell lines (4), have been shown to enter normal thymus. Peripheral B and T cells are also known to enter thymuses of autoimmune (NZB \times SJL) mice (5) and preleukemic AKR mice (unpublished observation). However, studies to identify entry of normal peripheral T lymphocytes into the thymus have produced conflicting results (2, 3, 6-9).

We have characterized a lymphocyte transfer technique using Thy-1 congenic mice that allows following of Thy-1 allotypically marked peripheral LN T cells up to several months in normal, unirradiated host mice under apparently physiologic conditions (10). We use this transfer technique here to directly demonstrate the entry of rare peripheral T lymphocytes into the normal mouse thymus, and to study their surface phenotype and microenvironmental location.

Materials and Methods

Lymphocyte Transfer. Transfers were performed as described (10) with 6-8-wk-old inbred congenic C57Bl/Ka Thy-1.1 and C57Bl/Ka Thy-1.2 mice. Each host mouse received 5×10^7 i.v. LN T cells as $\sim 10^8$ unfractionated lymphocytes. For mice killed 3 or 24 h after transfer, the thymus was briefly perfused in situ via the left ventricle with cold saline to remove intravascular lymphocytes.

Suspension Immunofluorescence (IF) Staining. Two-color stains were done using techniques and reagents as described (10), including mAb MEL-14 (anti-lymphocyte homing receptor [gp90] for peripheral LN high endothelial venules [HEV]) (11). Cells were visually examined by fluorescence microscopy and the percentage of total cells and donor Thy-1-type cells reactive with each antibody was determined. At least 100 cells/antibody per mouse were counted for both total cells and donor T cells.

Tissue Section Immunoperoxidase (IP) Staining and Evaluation. At least one lobe of thymus and one peripheral LN from each host mouse were frozen, cut, stained, and evaluated as described (10). The percent of donor T cells in the T cell population of the thymic medulla, thymic cortex, or LN paracortex was determined by counting donor T cells and host T cells

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in that microenvironment on sequential stained sections (10). A minimum of 10,000 cells per thymic microenvironment and 1,000 cells per LN paracortex were counted per mouse.

In one experiment in which host mice received various numbers of donor lymphocytes, ranging from 10^8 down to 6×10^6 , the number of donor T cells per square millimeter of host thymic medulla was determined using morphometric analysis (10). For each mouse, at least eight sections of thymus, with a minimum total medullary area of 10.4 mm^2 , were analyzed.

Results

Rare Peripheral T Cells Enter the Thymus and Are Localized to the Medulla. Thymuses and LN from 82 mice, ranging from 3 h to 24 wk after transfer of Thy-1 congenic LN cells, were examined by tissue section staining for both donor and host Thy-1. All thymuses contained donor peripheral T cells scattered individually throughout the medulla with extremely rare cells in the cortex (Fig. 1). Donor T cells in LN exhibited the same microenvironmental and immunophenotypic distribution as host T cells (10). There was no staining with antibody against donor type Thy-1 in thymuses and LN of noninjected control mice.

10 mice taken from 2 d to 24 wk after transfer were chosen for quantitative analysis of donor Thy-1-type cells in thymus and LN on sequential sections (Table I).

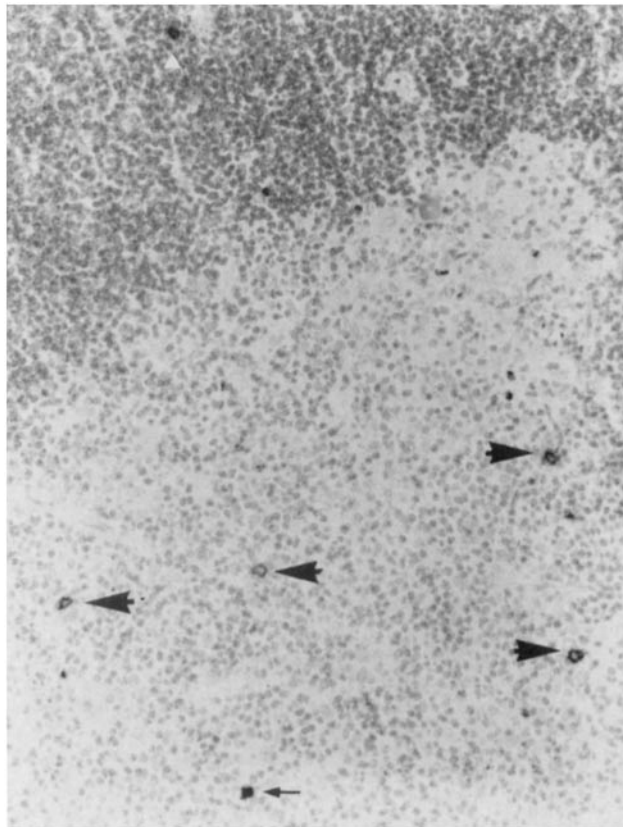


FIGURE 1. Frozen section of thymus from C57Bl/Ka Thy-1.1 mouse 10 d after transfer of Thy-1 congenic LN cells. Large black arrows identify four donor T cells in the medulla (lower part of figure). This field also contains one donor T cell (*white arrowhead*) in the cortex (upper part of figure); most fields of cortex contained no such cells. Cells with endogenous peroxidase activity (*small black arrow*) also stained on the negative control slides (immunoperoxidase with methylene blue; $\times 400$).

TABLE I
Donor T Cells Enter and Persist in Host Mouse Thymic Medulla

Time after transfer (No. of mice)	Percent donor T cells/total T cells		
	Thymic medulla	Thymic cortex	Lymph node paracortex
Day 2 (2)	0.2	0.01	11
Day 10 (1)	0.3	0	11
Week 2 (2)	0.2	0.005	11
Week 6 (1)	0.2	0.01	9
Week 12 (2)	0.3	0.005	6
Week 24 (2)	0.3	0.005	3

Host C57B1/Ka mice received 5×10^7 peripheral LN T cells from Thy-1 congenic donors. Values given are means. SD was <0.07 for thymic medulla, <0.01 for thymic cortex, and <0.7 for LN paracortex.

At all time points, the vast majority of donor T cells in the thymus were located in the medulla. The percentage of donor T cells in the total T cell population of the medulla remained relatively stable over time. In contrast, there was a gradual decrease in the percent of donor T cells in the T cell population of the LN paracortex with time, as previously noted (10).

It is possible that transfer of 10^8 lymphocytes might exceed normal thymic exclusion mechanisms and permit nonphysiologic entry of peripheral cells into the thymus. To address this possibility, in one experiment, host mice received variable numbers of Thy-1 congenic lymphocytes. Analysis of host thymuses 1 wk after transfer showed a linear decrease in the number of donor T cells in the medulla with a decrease in the number of cells transferred. The numbers of donor T cells/mm² of medulla (mean \pm SD; three mice per group) were 66 ± 7.4 , 29 ± 5.9 , 18 ± 3.5 , 6.5 ± 1.6 , and 4.2 ± 0.92 , respectively, as the number of transferred T cells decreased by twofold dilutions from 10^8 to 6×10^6 . For comparison to data in Table I, donor T cells were $0.5 \pm 0.1\%$ of lymphocytes in the thymic medulla of mice that received 10^8 cells. These data indicate that peripheral T cell traffic to the thymus is physiologic, rather than merely an "overload" phenomenon. In addition, we have observed donor T cells in thymic medulla after intraperitoneal injection of cells (data not shown) that would be expected to result in more gradual entry of transferred cells into the peripheral recirculating pool.

Peripheral T Cells that Enter the Thymus Express a Generally Stable Mature Phenotype. Thymic cell suspensions from 11 mice were examined with double-label IF stains for a variety of cell antigens (Table II). Since donor T lymphocytes were $<0.05\%$ of cells in the thymus (data not shown), the phenotype of the total cells is essentially that of host thymocytes alone. The phenotype of the host thymocytes was compatible with that of normal cortical thymocytes, predominantly Lyt-2⁺, L3T4⁺, PNA^{hi}, Ly-1^{lo}, and MEL-14^{lo} (12-14). The donor T cells in the thymus differed markedly from the host thymocytes, exhibiting as a population a phenotype of Ly-1^{hi}, MEL-14^{lo}, PNA^{lo}, with approximately two thirds of the cells L3T4⁺, and one third Lyt-2⁺. In six mice examined, $>90\%$ of donor T cells in the thymus also reacted with a mix of anti-Lyt-2 and anti-L3T4 (data not shown), indicating that these cells have a mature "single positive" phenotype. The percentage of donor T cells that were MEL-

TABLE II
Immunophenotypes of Total Cells and Donor T Cells in Host Mouse Thymuses

Time after transfer (No. of mice)	Cell type	LY-1		LYT-2		L3T4		PNA		MEL-14	
		hi	lo	Positive	Negative	Positive	Negative	hi	lo	hi	lo
3 h (4)	Total cells	ND	ND	90	10	88	12	ND	ND	2	98
	Donor T cells	ND	ND	48	52	46	54	ND	ND	17	83
24 h (2)	Total cells	ND	ND	ND	ND	ND	ND	ND	ND	1	99
	Donor T cells	ND	ND	ND	ND	ND	ND	ND	ND	34	66
2 wk (2)	Total cells	18	82	88	12	87	13	90	10	2	98
	Donor T cells	72	28	41	59	63	37	4	96	34	66
6 wk (1)	Total cells	24	76	90	10	78	22	81	19	1	99
	Donor T cells	76	24	36	64	76	24	2	98	10	90
12 wk (2)	Total cells	18	82	88	12	81	19	84	16	0	100
	Donor T cells	58	42	34	66	58	42	6	94	10	90
Mean	Total cells	19	81	89	11	85	15	86	14	1	99
	Donor T cells	67	33	42	58	56	44	4	96	21	79

Host C57B1/Ka Thy-1.1 mice received 5×10^7 peripheral LN T cells from C57B1/Ka Thy-1.2 donor mice. A thymus suspension was stained with anti-Thy-1.2 (for donor T cells) and either anti-Ly-1 (CD-5), anti-Lyt-2 (CD-8), anti-L3T4 (CD-4), MEL-14, or PNA using a double-label IF technique. Values given are mean percents. SD was <12 for each time point and <13 for the mean of all mice. SD for MEL-14 on donor T cells was 5.5 at 3 h, <2.8 at other time points, and 11.2 for the mean of all mice.

14^{hi} varied widely (8–36%) but was always greater than that in the total cell population. The phenotype of the donor T cells remained stable over time except for the number of MEL-14^{hi} cells that decreased somewhat with time (Table II).

Discussion

Previous studies designed to identify the entry of normal peripheral lymphocytes into thymus have produced conflicting results. For example, studies using chromosomal or radioactive markers have failed to demonstrate entry of peripheral lymphocytes into mouse thymus (3, 6). Conversely, rare thoracic duct lymphocytes and antigen-activated peripheral LN cells have been shown to enter the thymus (2, 7). In these studies, the number of T vs. B cells entering the thymus could not be determined. Our findings agree with functional data indicating recirculation of rare antigen-reactive peripheral T cells to thymus of normal mice (8) and to thymus of rats after experimental allergic encephalomyelitis (9).

Although progenitor cells are known to migrate into the thymus, it is unlikely that donor T cells seen in the host thymus are derived from transferred progenitor cells as: (a) thymocytes derived from transferred progenitor cells exhibit a variety of patterns of distribution in the thymus (15); (b) there is no significant population of donor cells of cortical thymocyte phenotype; (c) LN have not been shown to contain thymic progenitor cells (16); and (d) unirradiated thymus is capable of receiving only very rare stem cells (1).

The MEL-14 antigen, found on the surface of most mouse peripheral lymphocytes, is important in the binding of recirculating lymphocytes to LN HEV (11).

In normal thymus, a minority of cells expresses high levels of MEL-14. The location of these cells is a subject of debate as tissue section staining reveals positive cells scattered in the cortex but suspension staining demonstrates most MEL-14^{hi} cells to bear a medullary phenotype (13, 14). Although it has been proposed that MEL-14^{hi} thymocytes represent thymic progeny poised for emigration (13), at least some such cells must instead be T cells that have reentered thymus from the periphery. The increase over time in MEL-14^{lo} donor T cells may indicate their selection from the pool of mixed phenotype cells circulating through the thymus, or a microenvironmentally induced phenotypic change in cells selected on another basis for long-term residency.

The functional significance of peripheral T lymphocytes in thymus is unclear. Previous studies have shown antigen-activated peripheral T cells, or cells from recently antigen-activated T cell lines, can enter thymus and remain there for several months (4, 8, 9). As our experiments included transferred cells from LN, such as cervical and mesenteric, which contain many antigen-activated lymphocytes, it is possible that activation of peripheral T cells plays a role in their ability to enter the thymus. These thymic immigrants do not appear to represent a functionally defined subset of mature T cells based on their surface L3T4 and Lyt-2 phenotypes. Our data indicate that the peripherally derived thymic cytotoxic lymphocytes identified by Fink et al. (8) must reside in the medulla, the compartment that also contains potent APC (interdigitating cells) and access to peripherally circulating soluble antigens (17). Thus the thymic medulla exhibits characteristics of the T cell domain of a peripheral lymphoid organ and is potentially a site of immune reactions. This may have implications for autoimmune reactions, such as found in myasthenia gravis (18) and in NZB mice (19), in which there is thymic lymphoid hyperplasia. In addition, access of peripheral T cells to the thymic medulla may play an as yet undefined role in development of immunologic memory and in maturation and differentiation of thymocytes.

Summary

The traffic of T cells between the thymus and peripheral lymphoid organs is generally thought to be unidirectional. Using a technique of lymphocyte transfer between Thy-1 congenic mice, we demonstrate here the entry of rare peripheral lymph node T cells into the normal mouse thymus. At time points from 3 h to 24 wk after transfer, donor peripheral T cells were present in the host thymus, mainly as scattered single cells confined to the medulla. At 2 wk after transfer, donor T cells constituted 0.2% of the medullary thymocytes (compared with 11% of the peripheral lymph node T cells). As a population, these cells exhibited a stable mature immunophenotype (Ly-1^{hi}, PNA^{lo}, and mixed L3T4⁺ and Lyt-2⁺). A minority of the donor T cells expressed high levels of the MEL-14 "homing receptor". The thymic medulla thus exhibits features of a peripheral lymphoid organ but differs in its low rate of turnover of recirculating T cells.

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