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Traffic of Peripheral B and T Lymphocytes to Hyperplastic, Preneoplastic Thymuses of AKR Mice

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AKR mice develop hyperplasia of the thymus before the development of retrovirus-associated lymphoma at that site. This hyperplasia, first detectable in AKR/J mice by 4 weeks of age and in AKR/C mice by 4 to 5 months of age, is characterized by an enlarged thymic medulla that contains T and B lymphocytes. In contrast to the general population of thymocytes, most of these T and B lymphocytes have a mature immunophenotype that includes expression of high levels of the MEL-14–defined (gp90) 'homing receptor' for peripheral lymph node high endothelial venules. In vivo ***homing studies reveal a marked increase in traffic of peripheral lymphocytes (T more than B) to the hyperplastic thymuses of old AKR mice as compared to histologically normal thymuses of age-matched BALB/c and C57BL/Ka mice or young AKR mice. These changes correlate chronologically with changes in retrovirus antigen expression in AKR thymuses and suggest a role for the traffic of lymphocytes from the periphery to the thymus in response to local antigenic stimulation in the pathogenesis of thymic hyperplasia in AKR mice. (Am J Pathol 1991, 138:1015–1025).***

Virtually all AKR mice develop thymic lymphoma with increasing age.[1] A variety of changes have been described in the preneoplastic AKR thymus, including the expression of endogenous retroviruses,[2] the development of a recombinant, leukemogenic retrovirus,[3,4] phenotypic changes in the thymocyte population,[5–7] and thymic hyperplasia.[1] The thymic hyperplasia is characterized by an enlarged medulla that contains lymphocyte follicles. These architectural changes as well as some of the reported phenotypic changes suggest that peripheral B and T cells make a significant contribution to the microenvironment of the hyperplastic, preneoplastic AKR thymus.

Most peripheral B and T lymphocytes migrate continuously throughout the body, trafficking from blood through lymphoid tissues to lymph and back to blood.[8] The thymus generally is thought to be excluded from the peripheral lymphocyte recirculation route. We recently showed, however, that small numbers of lymph node (LN) T and B lymphocytes traffic to normal thymuses of adult BALB/c and C57BL/Ka mice.[9,10] In addition Dumont et al[11] demonstrated increased traffic of lymphocytes into the hyperplastic thymuses of aging autoimmune (NZB × SJL) F1 female mice as compared to normal thymuses of younger mice. In this study, we characterize the migration of peripheral B and T lymphocytes to hyperplastic preneoplastic thymuses of aging AKR mice and describe the surface phenotypes of these lymphocytes. The observations contrast with those seen in histologically normal thymuses of age-matched BALB/c and C57BL/Ka mice and young AKR mice.

Materials and Methods

Mice

Five-week-old AKR/J and AKR/C mice were purchased from Jackson Laboratories (Bar Harbor, ME) and Cumberland Farms (Cumberland, TN), respectively, housed in our animal facility under standard conditions, and used at ages 7 to 31 weeks. Mice bred in our facility from the above-mentioned stock were used in selected experiments at ages 1 day to 31 weeks. All experiments involved female mice unless otherwise stated.

Female BALB/c mice, obtained from the Institute for Medical Research (San Jose, CA) or bred in our animal facility, and C57BL/Ka Thy-1.1 and C57BL/Ka Thy-1.2

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mice, bred in our facility, were used at ages ranging from 1 to 105 weeks.

Unmanipulated AKR/J, AKR/C, BALB/c, and C57BL/Ka Thy-1.1 mice were killed and the thymuses were dissected free of the parathymic LN, removed, and weighed. Cell suspensions from one half of each thymic lobe were prepared in Hank's balanced salt solution with 2% newborn calf serum, pH 7.4, plus 0.1% azide. The remaining thymic tissue was frozen in optimal cutting temperature (OCT) compound (Miles Laboratories, Naperville, IL) or fixed in formalin and embedded in paraffin. In selected mice, peripheral lymph nodes (PLN) were also removed, then suspended and/or frozen.

Frozen sections of lymphoid organs were used for immunoperoxidase studies as described below. Adjacent sections stained with hematoxylin and eosin (H&E), as well as H&E-stained sections of paraffin-embedded tissue were evaluated using standard histologic criteria. Cell suspensions were used for immunofluorescence (IF) studies as described below.

Monoclonal Antibodies

The following monoclonal antibodies (MAb) were prepared in our laboratory and conjugated with biotin, fluorescein (FITC), or allophycocyanin (APC) as described[12]: MEL-14 (anti-lymphocyte homing receptor for PLN),[13] RA3-2C2 (anti-B220; CD45RA), RA3-6B2 (anti-B220; CD45R),[14] RA3-3A1 (anti-B220; CD45RA),[15] 10-3.6 (anti–I-A^k), 10-4.22 (anti-mouse IgD, allotype a),[16] Bet-II (anti-mouse IgM),[17] 19XE5 (anti–Thy-1.1, allotype specific),[18] 53-2.1 (anti–Thy-1.2, allotype specific), 53-6.7 (CD8), 53-7.3 (CD5),[19] GK1.5 (CD4),[20] M1/70 (anti–Mac-1; CD11b),[21] and 7D4 (anti-Tac interleukin-2 receptor; CD25).[22] C2F2 (anti-transferrin receptor; CD71) was a gift from Dr. John D. Kemp.[23] All are rat MAbs except for mouse MAb 19XE5, 10-4.22 and 10-3.6.

Lymphocyte Transfer

Suspended LN cells from 6- to 8-week-old AKR, BALB/c, or C57BL/Ka mice were transferred intravenously into host mice ranging in age from 7 to 31 weeks (AKR) or 7 to 97 weeks (BALB/c and C57BL/Ka). In most experiments, host mice received 5×10^7 T cells as approximately 7 to 9×10^7 total cells from Thy-1 congenic donors.[24] In a few experiments, host mice received 5×10^7 or 1×10^8 FITC- or rhodamine-labeled cells from syngeneic donors.[25] In each experiment, a small sample of the donor cell suspension was reserved for IF stains.

Host mice were killed 1 hour to 1 week after transfer. One half of each of the following lymphoid organs was taken for suspension and the remaining half frozen: thymus, spleen, PLN, mesenteric LN (MLN), and Peyer's patches (PP). In selected experiments, blood was collected in heparinized saline and lymphocytes isolated by Lymphocyte-M density centrifugation (Cedarlane Laboratories, Hornby, Ontario, Canada). Suspensions and frozen tissue were used for IF or immunoperoxidase stains as described below.

Tissue Section Immunoperoxidase Staining and Evaluation

Frozen sections of lymphoid organs were stained and evaluated as previously described using one of the following protocols: 1) biotin-conjugated MAb, peanut agglutinin lectin (PNA) (Vector Laboratories, Burlingame, CA), or polyclonal goat anti-mouse immunoglobulin (Ig) (Sigma, St. Louis, MO) followed by peroxidase-avidin (Vector); or 2) unconjugated rat MAb followed by biotin-conjugated rabbit anti-rat Ig (Vector) in 5% normal mouse serum (NMS) and then peroxidase-avidin. Peroxidase activity was revealed by incubation with 3,3′-diaminobenzidine/hydrogen peroxide solution followed by stain-intensifying copper sulfate.[24] As negative controls, primary antibodies were replaced with species, isotype, and conjugation matched irrelevant MAb or phosphate buffered saline (PBS).

Suspension IF Staining and Analysis

Cell suspensions from lymphoid tissues of unmanipulated AKR, BALB/c, or C57BL/Ka Thy-1.1 mice were stained for single-color IF as described using biotin-conjugated MAb or polyclonal anti-mouse Ig followed by FITC-avidin (Becton-Dickinson, Mountain View, CA).[24] For two-color IF, one of the following protocols was used: 1) biotin–RA3-2C2, RA3-6B2, or polyclonal anti-mouse Ig (in 10% normal rat serum (NRS)) followed by Texas Red-avidin admixed with either FITC-Bet-II, 10-4.22, 10-3.6, CD5, or CD11b; 2) biotin-anti–Thy-1 followed by Texas Red-avidin plus FITC-CD4, CD8, or CD5; or 3) sequential incubation with MEL-14; FITC-rabbit anti-rat IgG (Zymed Laboratories, South San Francisco, CA) in 5% NMS; 10% NRS; biotin–RA3-2C2, RA3-6B2, anti–Thy-1, or polyclonal anti-mouse Ig (in 10% NRS); and Texas Red-avidin. Three-color IF stains were performed by incubating cells with biotin-anti–Thy-1 or biotin–MEL-14 followed by APC-CD4 admixed with FITC-CD8 and Texas Red-avidin; or biotin–10-4.22 followed by APC-Bet-II admixed with FITC-RA3-2C2 or RA3-6B2 or polyclonal anti-mouse Ig (in 10% NRS) and Texas Red-avidin. As negative controls, primary antibodies were replaced with species-,

isotype-, and conjugation-matched irrelevant MAb or PBS. Following the final wash, cells were examined by fluorescence microscopy or incubated with propidium iodide and used for fluorescence-activated cell sorter (FACS) analysis as previously described.[24] Data are presented graphically as histograms or 10% probability contour maps, or numerically as percentage of cells reacting with a specific antibody minus percentage of cells reacting with negative control antibody.

Suspensions of lymphoid organs from mice that had received transfers of Thy-1 congenic lymphocytes were stained using one of the following protocols: 1) FITC–anti-donor allotype Thy-1; or 2) biotin–MEL-14, CD4, CD5, CD8, C2F2, 10-3.6, or 7D4, followed by FITC–anti-donor Thy-1 admixed with Texas Red-avidin; or 3) biotin–anti-donor Thy-1 followed by APC-CD4 admixed with FITC-CD8 and Texas Red-avidin. Suspensions from mice that had received fluorochrome-labeled cells were examined unstained or stained with biotin-conjugated RA3-2C2, RA3-6B2, polyclonal anti-mouse Ig, anti-donor Thy-1, or MEL-14 followed by Texas Red-avidin (for FITC-labeled donor cells) or FITC-avidin (for rhodamine-labeled donor cells). Suspensions were examined by fluorescence microscopy or FACS and the number and phenotype of donor cells in host lymphoid organs was determined.[24]

Results

Hyperplasia of the Preneoplastic AKR Thymus

AKR mice develop thymic hyperplasia, characterized histologically by an enlarged medulla that contains lymphoid follicles, before the development of lymphoma at that site.[1] To study the changes that occur in the preneoplastic AKR thymus, we examined thymuses from AKR mice ranging in age from 1 day to 31 weeks for changes in histology, immunohistology, and lymphocyte subset phenotypes. Thirty-one weeks was chosen as the cut-off age for this study because we first saw histologic evidence of lymphoma in thymuses from 34-week-old mice (1 of 6 mice). Unless otherwise noted, all AKR mice 7 weeks of age or older of each strain were from a single large cohort of female mice obtained from the breeders. Thymuses of age-matched and older BALB/c and C57BL/Ka mice were examined for comparison.

Three major changes in the immunohistology and the overall phenotype of the lymphocyte subsets in the AKR thymus occurred with age, the first being an increase in the relative number of B cells. As illustrated in Figure 1, a very small percentage of the cells found in histologically normal thymuses of BALB/c mice were B cells and this

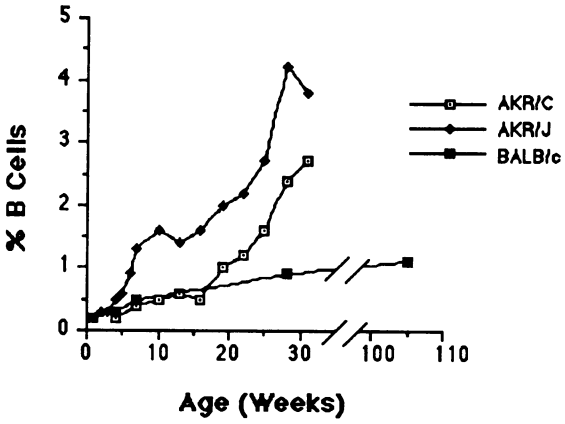

Figure 1. *Thymic B cells increase in percentage with increasing age in AKR mice. Data shown were obtained using suspension IF staining with MAb RA3-6B2. Similar results were obtained when B cells were defined by reactivity with polyclonal anti-mouse Ig or by strong reactivity with MAb RA3-2C2 or RA3-3A1. Each value is the mean of three mice. SEM were <0.3 for all BALB/c, 22 weeks or younger AKR/C, and 19 weeks or younger AKR/J mice; <0.6 for all other mice, except 31-week-old AKR/J mice where SEM = 1.2.*

percentage remained relatively stable throughout adult life. The numbers of B cells in C57BL/Ka thymuses were similar to those in BALB/c thymuses (data not shown). The percentage of B cells in the AKR/J thymuses exceeded that in BALB/c thymuses by 4 weeks of age and continued to increase with age. In contrast to AKR/J mice, AKR/C mice did not show a noticeable increase in the number of intrathymic B cells until about 19 weeks of age. Similar age-related changes in the numbers of intrathymic B cells were noted in thymuses of male and female AKR mice of both strains born and raised in our animal colony (data not shown).

The phenotypes of these intrathymic B cells were examined in 7-, 16-, and 31-week-old AKR/J and 7- and 25-week-old AKR/C mice, and compared to the phenotypes of LN B cells from these mice and 6- to 8-week-old BALB/c mice. The overall phenotypes of the AKR intrathymic B cells were identical to those of LN B cells: RA3-6B2^{hi}, RA3-2C2^{hi}, IgM$^{lo\ to\ medium}$, IgD^{hi} with a subpopulation IgD^{neg} (Figure 2), I-A^{+}, CD5^{-}, CD11b^{-}, and bimodal distribution of MEL-14 (see below). This phenotype also was found on B cells from histologically normal thymuses of BALB/c and C57BL/Ka mice.[10] Tissue section immunoperoxidase studies on histologically normal thymuses of young AKR mice revealed that these B cells were scattered individually throughout medulla and cortex. This pattern of distribution was identical to that seen in thymuses of BALB/c or C57BL/Ka mice.[10] In contrast, in thymuses of old AKR mice showing histologic evidence of hyperplasia, the majority of the intrathymic B cells resided in primary follicles in the region of the medulla or corticomedullary junction (CMJ) (Figure 3). Germinal centers, characterized immunohistologically by a central core of PNA^{+} Ig^{lo} large lymphocytes, were seen

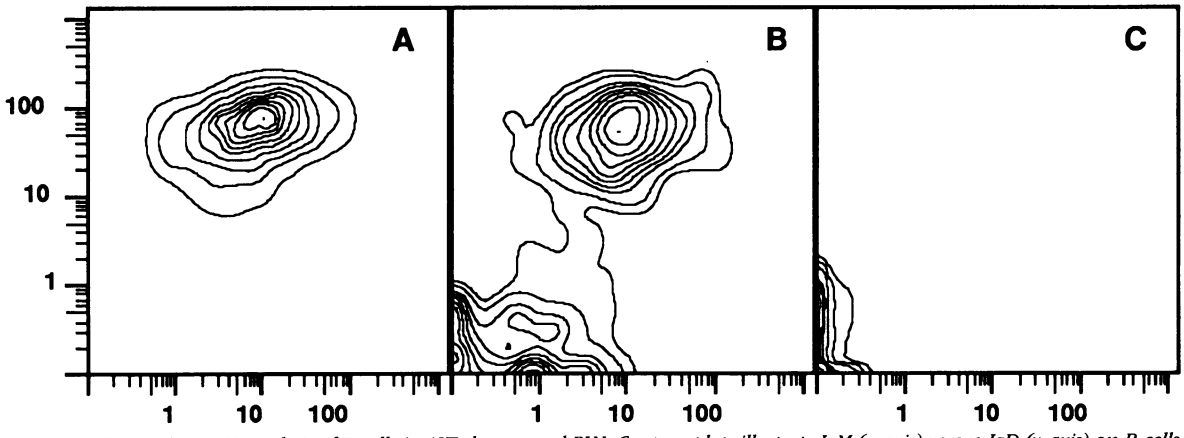

Figure 2. *Three-color FACS analysis of B cells in AKR thymus and PLN. Contour plots illustrate IgM (x axis)* versus *IgD (y axis) on B cells (RA3-6B2$^+$) from PLN* (**A**) *and hyperplastic thymus* (**B**) *of a 31-week-old AKR/J mouse.* **C** *shows negative controls on thymus. The majority of B cells in PLN display the IgM$^{lo/medium}$ IgD^{hi} phenotype of conventional B cells. A similar staining profile was obtained on PLN B cells from 6- to 8-week-old BALB/c mice. The majority of B cells in AKR thymus share the IgM IgD phenotypes of PLN B cells while a subpopulation of thymic B cells is IgM$^{lo/medium}$ IgD^{neg}. The B cells in PLN and thymus are also negative for CD5 and CD11b (data not shown).*

in sections of thymuses from only 3 of the 75 AKR mice illustrated in Figure 1. The germinal centers comprised less than 20% of the thymic B follicles in each of these three mice (AKR/J 19 weeks old, AKR/J 28 weeks old, and AKR/C 31 weeks old).

Second there was an increase with age in the percentage of AKR thymic T cells with the 'single positive' phenotype (CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$) characteristic of mature thymocytes and peripheral T cells.[26] This increase was of greater magnitude than that seen in thymuses of aged-matched BALB/c mice (Table 1).

Third the percentage of AKR thymic lymphocytes expressing high levels of the MEL-14 PLN 'homing receptor' increased with age. For this study, lymphocytes express-

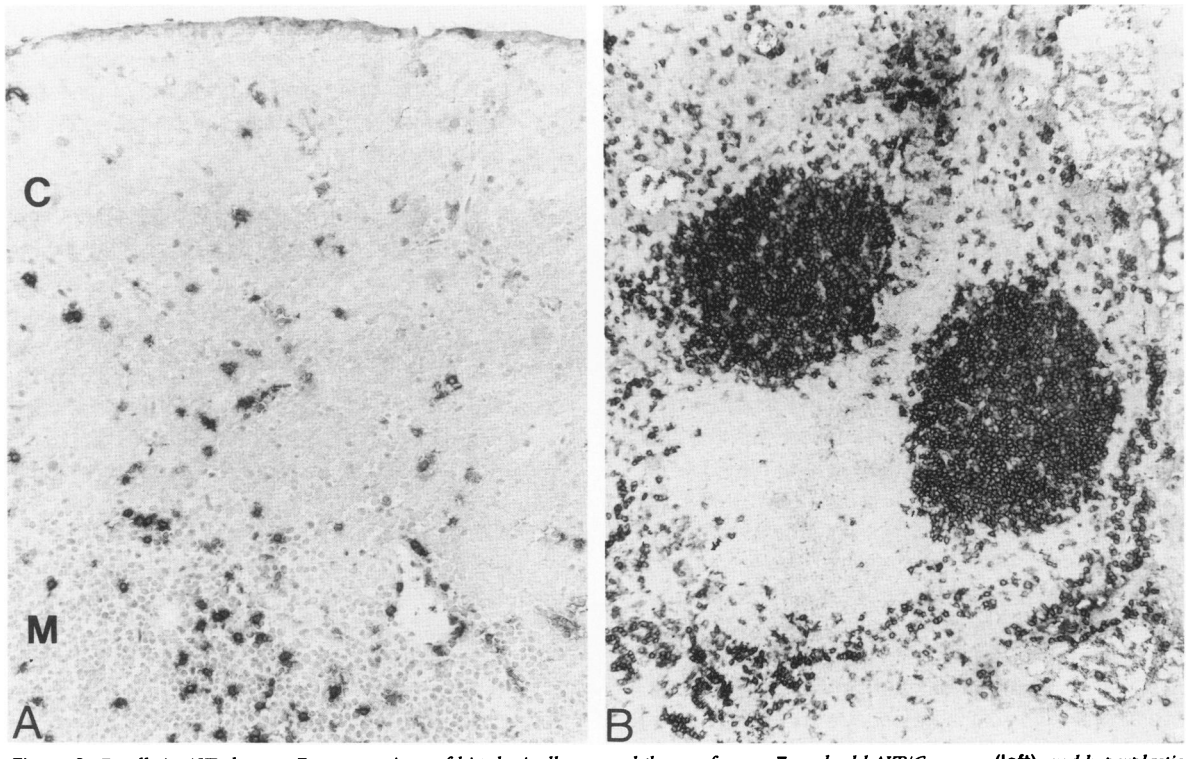

Figure 3. *B cells in AKR thymus. Frozen sections of histologically normal thymus from a 7-week-old AKR/C mouse* (**left**) *and hyperplastic thymus from a 28-week-old AKR/C mouse* (**right**) *were stained with MAb RA3-6B2 using an immunoperoxidase technique. Small numbers of B cells are scattered singly throughout cortex* (**C**) *and medulla* (**M**) *of thymus from the 7-week-old AKR/C* (**left**). *Similar numbers and microenvironmental distribution of B cells were seen in thymuses of BALB/c and C57BL/Ka mice ranging in age from 7 to 105 weeks. In contrast, many B-cell follicles are found in the medullary region of the AKR hyperplastic thymus (right) (immunoperoxidase, ×40).*

Table 1. *B- and T-lymphocyte Numbers and Immunophenotypes in Normal and Hyperplastic Thymuses*

Strain–Age (weeks)	Organ	Weight (g)	% B cells	% Single positive T cells	% MELhi of Total cells	% MELhi of B cells	% MELhi of Single positive T cells
BALB/c–7	Thymus	0.12 ± 0.01	0.5 ± 0.2	12 ± 1	23 ± 2	41 ± 1	54 ± 3
	PLN	ND	ND	ND	72 ± 4	76 ± 3	70 ± 4
BALB/c–28	Thymus	0.04 ± 0.01	0.8 ± 0.2	15 ± 2	24 ± 3	38 ± 3	59 ± 1
	PLN	ND	ND	ND	66 ± 2	69 ± 3	65 ± 1
BALB/c–52	Thymus	0.02 ± 0.01	1.2 ± 0.3	20 ± 3	29 ± 4	48 ± 3	62 ± 3
	PLN	ND	ND	ND	74 ± 3	73 ± 4	75 ± 4
AKR/C–7	Thymus	0.13 ± 0.01	0.4 ± 0.1	14 ± 2	26 ± 2	43 ± 3	53 ± 2
	PLN	ND	ND	ND	68 ± 5	70 ± 2	68 ± 3
AKR/C–28	Thymus	0.08 ± 0.02	2.4 ± 0.3	27 ± 4	35 ± 5	67 ± 6	69 ± 4
	PLN	ND	ND	ND	68 ± 7	65 ± 7	69 ± 5
AKR/J–28	Thymus	0.09 ± 0.02	4.3 ± 0.8	30 ± 6	38 ± 8	80 ± 8	75 ± 9
	PLN	ND	ND	ND	71 ± 4	76 ± 5	70 ± 5

ing high surface levels of the MEL-14 'homing receptor' (MEL-14^{hi}) *versus* those with little or no receptor (MEL-14$^{lo/neg}$) were defined by the natural separation between these two populations of lymphocytes in LN (Figure 4). As illustrated in Table 1, an increase in percentage MEL-14^{hi} AKR thymic cells with age was evident in both the B-cell and the 'single positive' T-cell populations. In contrast, there was no significant change in the MEL-14 phenotype of the cells in the 'double positive' (CD4$^+$CD8$^+$) subpopulation of AKR thymic T cells during the same time period (data not shown). Tissue section immunohistology showed the majority of MEL-14^{hi} cells in histolog-

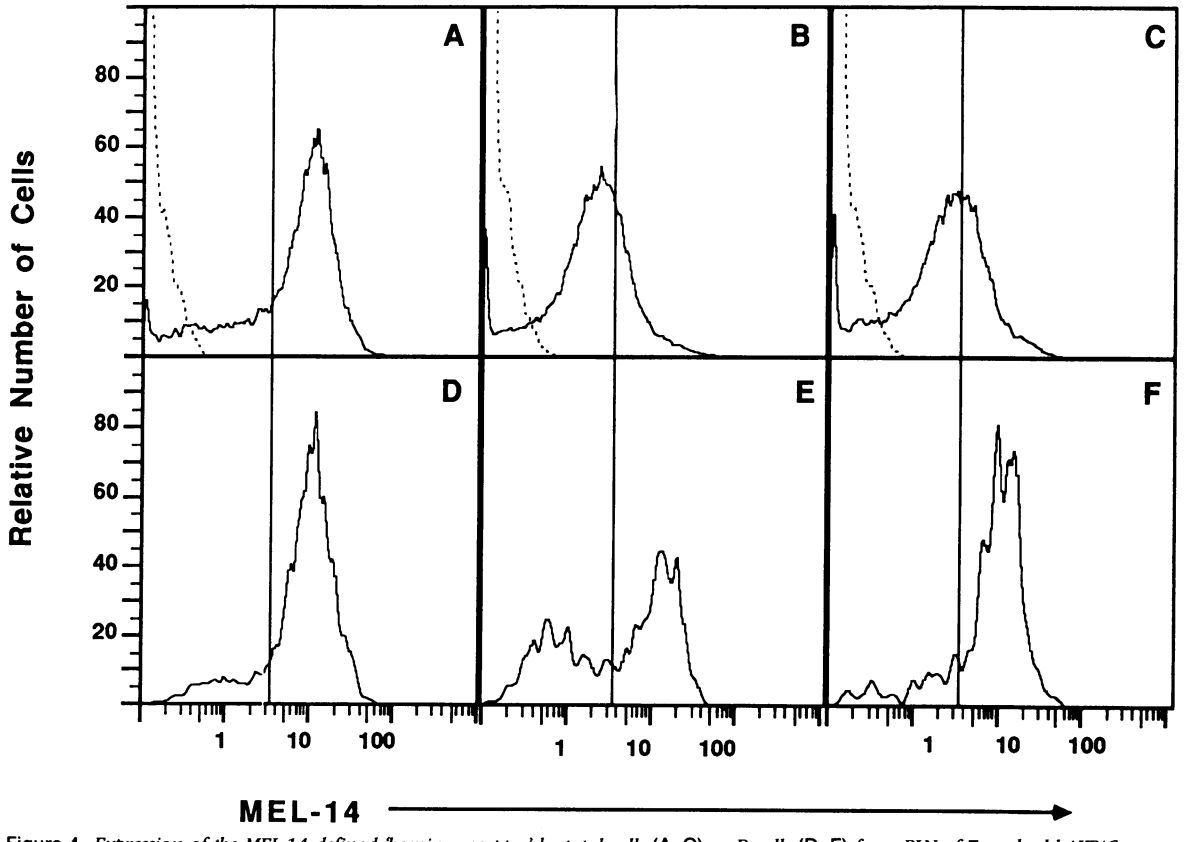

Figure 4. *Expression of the MEL-14 defined 'homing receptor' by total cells* (A–C) *or B cells* (D–F) *from PLN of 7-week-old AKR/C mouse* (A,D), *histologically normal thymus of 7-week-old AKR/C mouse* (B,E), *and hyperplastic thymus of 25-week-old AKR/C mouse* (C,F). *The majority of B and T lymphocytes* (A,D) *in PLN express high levels of MEL-14 (to the right of vertical line) with a small number of cells being MEL-14$^{lo/neg}$ (left of vertical line). Most cells in AKR thymus* (B,C) *are MEL-14$^{lo/neg}$. B cells in thymus from young AKR mouse show a bimodal distribution of MEL-14 expression* (E) *similar to that shown by B cells in BALB/c or C57BL/Ka thymuses. The majority of B cells in hyperplastic AKR thymus* (F) *are MEL-14^{hi}. Negative control stains are indicated by dotted lines.*

ically normal thymuses of BALB/c, C57BL/Ka, or young AKR mice to be in the cortex or at the corticomedullary junction, with very few such cells located in the medulla.[27,28] In contrast, the majority of the MEL-14^{hi} cells in hyperplastic AKR thymuses were located in and around the follicles in the medullary region (Figure 5).

Increased Traffic of Peripheral B and T Lymphocytes to the Hyperplastic AKR Thymus

The increase in intrathymic lymphocytes exhibiting a mature phenotype suggests that there may be an increase in traffic of peripheral lymphocytes to hyperplastic AKR thymuses as compared to histologically normal thymuses. We studied the peripheral T-cell traffic to the AKR thymus using a technique of lymphocyte transfer between Thy-1 congenic mice (AKR/J = Thy-1.1; AKR/C = Thy-1.2). This technique permits short- or long-term T-lymphocyte homing studies to be performed under relatively physiologic conditions.[24] As illustrated in Figure 6, only a small number of peripheral T cells migrated to thymuses of 7- to 16-week-old AKR/C mice, or to thymuses of 7- to 97-week-old C57BL/Ka mice. The traffic to AKR/C thymuses increased with recipient age, with a 10-fold increase between 7 and 31 weeks of age. Although there was already substantial peripheral T-cell traffic to the thymuses of 7-week-old AKR/J mice, this traffic also increased with age.

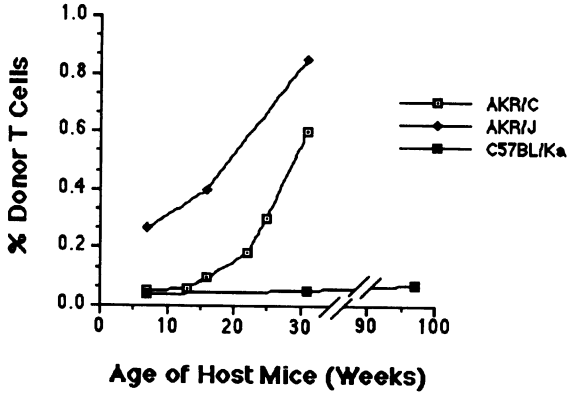

Figure 6. *Peripheral T-cell traffic to thymus increases with increasing age in AKR mice. Host mice of various ages received 5 × 10^7 LN T cells from 6- to 8-week-old Thy-1 congenic donors. Twenty-four hours after transfer, the numbers of donor T cells in host thymuses were determined. AKR host mice were from the same cohorts as those illustrated in Figure 1. Each value is the mean of at least five mice. SEM were <0.05 for all C57BL/Ka and 22 weeks or younger AKR/C mice and <0.1 for all other mice, except 31-week-old AKR/J mice where SEM = 0.3.*

Immunohistology revealed that the vast majority of these donor peripheral T cells in the thymuses of AKR and C57BL/Ka mice were located in the medulla. The donor T cells were scattered evenly throughout the thymic medulla in all C57BL/Ka and 7- to 16-week-old AKR/C mice examined. In hyperplastic thymuses of the older AKR/C and adult AKR/J mice, the donor T cells were distributed in the T-cell zones surrounding B-cell follicles in the medulla and CMJ.

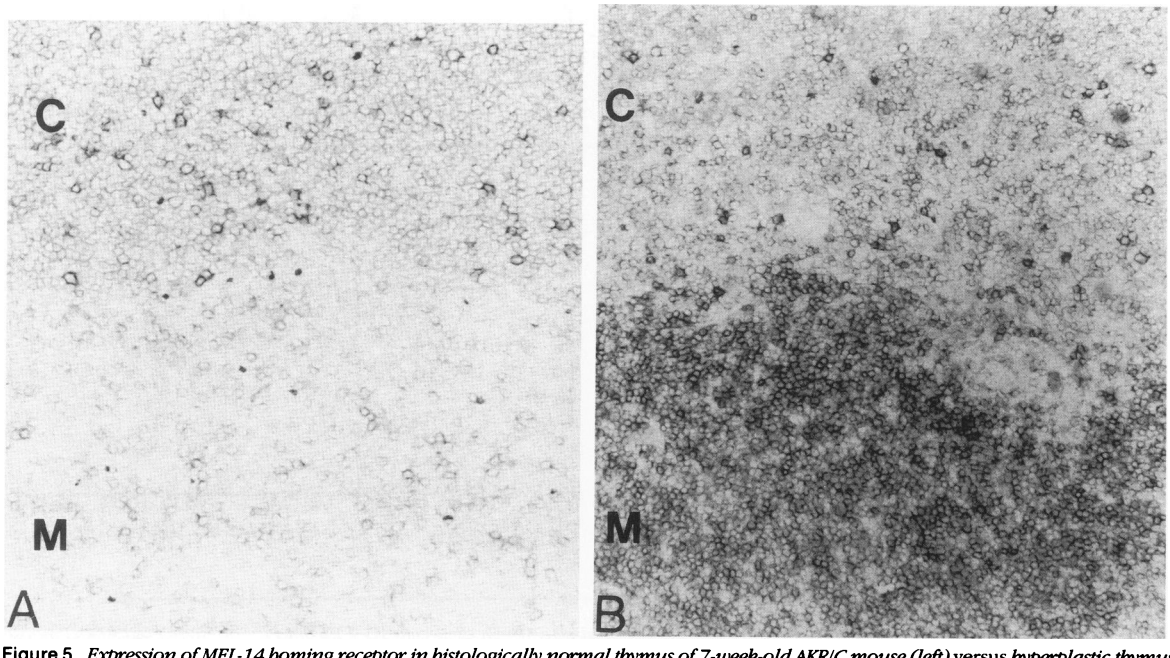

Figure 5. *Expression of MEL-14 homing receptor in histologically normal thymus of 7-week-old AKR/C mouse (left)* versus *hyperplastic thymus of 28-week-old AKR/C mouse (right). Cells in cortex* (C) *show a wide range of MEL-14 expression in both mice, similar to the pattern of expression seen in thymic cortex of BALB/c mice. Most cells in medulla (M) of histologically normal thymuses of young AKR mice (left) or adult BALB/c mice (not shown) appear MEL-14$^{lo/neg}$, while majority of medullary cells in hyperplastic AKR thymuses stain strongly with MEL-14 (right) (immunoperoxidase, ×40).*

The short-term traffic of both B and T peripheral lymphocytes to the thymus was examined by following FITC- or rhodamine-labeled transferred LN lymphocytes. Thymuses of AKR or BALB/c host mice were examined 1, 3, or 24 hours after transfer, and the numbers of donor B and T cells in each host thymus were determined. The number of peripheral lymphocytes trafficking to the AKR thymus increased with host age, with most of this increase due to an increase in T-cell traffic (Figure 7). For example, 3 hours after transfer, a mean of 0.03% of the total suspendable cells in thymuses of 7-week-old AKR/C host mice were donor B cells, while 0.1% of cells in 30-week-old AKR/C thymuses were donor B (threefold increase); at these two ages 0.06% and 0.6%, respectively, of thymic cells were donor T cells (10-fold increase) (n = 3 mice per group). In contrast, there was not a major age-related change in the numbers or B:T ratio of the peripheral cells migrating to thymuses of BALB/c mice (Figure 7).

Immunophenotype of T Cells that Traffic to AKR Thymus

The immunophenotype of the Thy-1 congenic donor peripheral T lymphocytes that traffic to the AKR thymus was determined by suspension IF staining followed by FACS analysis or fluorescence microscopy. At time points ranging from 1 hour to 1 week after transfer, these thymic emigrants exhibited a mature, resting immunophenotype: CD-5^{hi}, CD4$^+$CD8$^-$ or CD4$^-$CD8$^+$ ('single positives'), Tac$^-$, transferrin receptor$^-$, I-A$^-$, and a bimodal distribution of MEL-14 reactivity (Figure 8).

To determine if there was selective migration of T-cell subsets (CD4$^+$ *versus* CD8$^+$) to the AKR thymus, the ratio of CD4$^+$:CD8$^+$ donor T cells in the recipient organs was compared to that of the donor cell population. No selective migration of one subset over the other was seen to histologically normal thymuses of 7-week-old AKR/C or 7-, 30-, or 90-week-old C57BL/Ka mice (Figure 9). However there was preferential migration of CD4$^+$ over CD8$^+$ T cells to the hyperplastic thymuses of 30-week-old AKR/C mice. Three-color FACS analysis showed these donor T cells in the 30-week-old AKR/C thymuses to be 'single positives' (Figure 8).

The MEL-14 phenotype of the donor T cells in AKR host thymus was determined at various times after transfer and compared to that of the input cells in a fashion similar to that above. Data from representative experiments is presented in Figure 10. In 7-week-old AKR/C and 7-, 30-, and 90-week-old C57BL/Ka mice, the percentage of donor T cells in thymus that were MEL-14^{hi} was less than the percentage in the donor cell population. In contrast, the majority of T lymphocytes that migrated to the hyperplastic thymuses of 30-week-old AKR/C mice were MEL-14^{hi} (Figure 8).

Discussion

We demonstrate in this report that a marked increase in the entry and retention of peripheral B and T lymphocytes is an early abnormality in the preneoplastic AKR thymus. In the thymuses of very young mice (less than 4 weeks old for AKR/J and 19 weeks old for AKR/C), the patterns of recirculation are indistinguishable from the thymuses of normal (nonlymphomatous) strains of mice. By 4 weeks of age in AKR/J mice, however, there is already clear

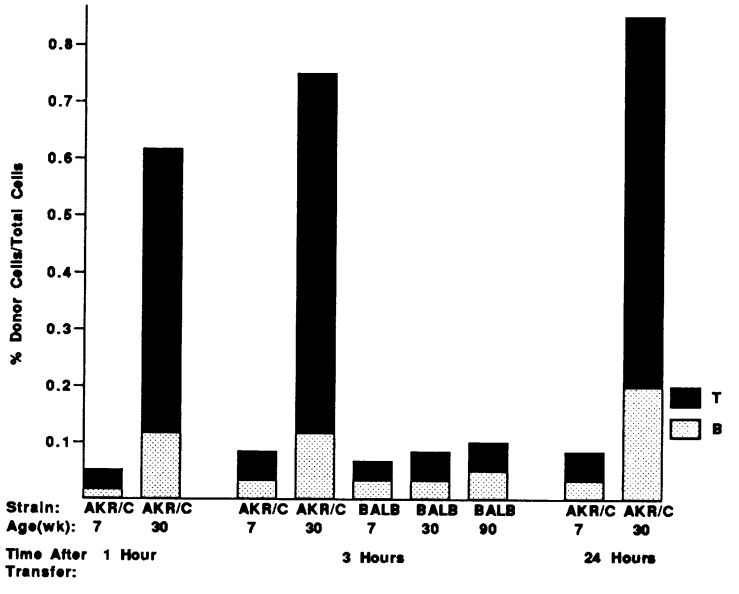

Figure 7. *Peripheral B- and T-cell traffic to thymus. The numbers of donor B cells (stippled bars) and donor T cells (solid bars) in host thymuses were determined 1, 3, or 24 hours after transfer of 1 × 10^8 rhodamine-labeled syngeneic LN lymphocytes. The transferred AKR donor cells were 35% B, 63% T, while BALB/c donor cells were 40% B, 56% T.*

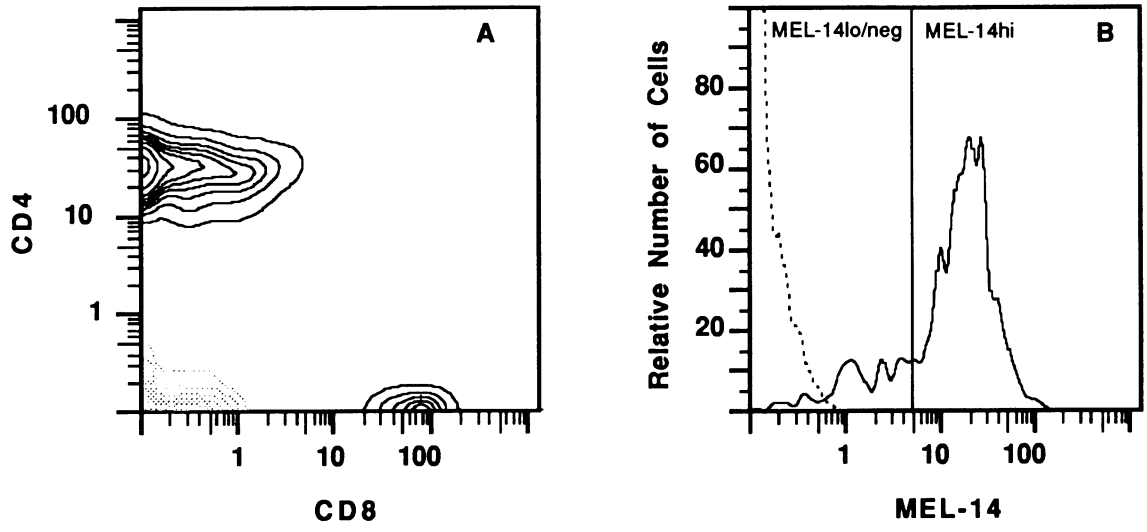

Figure 8. *Peripheral T cells that traffic to AKR thymus have mature immunophenotypes. Representative FACS analysis plots from one of the 30-week-old AKR/C mice from Figure 6 are gated to illustrate the phenotypes of the donor T cells in the host thymus.* A: *The donor T cells in host thymus are single positives.* B: *Most of the donor T cells are MEL-14^{hi}. Negative control stains on A and B are indicated by dotted lines.*

evidence of abnormalities in recirculation patterns. At this age, the percentage of thymic B cells already approaches the value seen in 1-year-old normal mice,[10] eventually reaching 4% in late preneoplastic mice. These cells bear surface phenotypes similar to peripheral B cells. At the same age, there is an increase in thymic T cells bearing 'single positive' ($CD4^+CD8^-$ or $CD4^-CD8^+$) phenotype, typical of mature, peripheral T cells. While both of these populations could have been generated intrathymically, as B-cell precursors have been demonstrated in the embryonic thymus[29] and 'single positive' T cells are a normal product of thymocyte differentiation,[26] our recirculation studies indicate that many of these cells must have entered from the periphery as mature cells. Both short- and long-term studies of transferred peripheral lymphocytes demonstrate a breakdown in the thymic barrier to peripheral cell entry that correlates chronologically with the appearance of increased numbers of cells bearing the mature phenotypes noted above.

The recirculation of lymphocytes to peripheral lymphoid organs is mediated by interactions between lymphocyte surface homing receptors and their ligands on endothelial cells.[30] Peripheral lymphocytes as a population express the peripheral lymph node homing receptor MEL-14 in a bimodal fashion, with most cells expressing it at high levels. Our previous studies demonstrated that in the thymus of normal mice, the B and T cells that re-

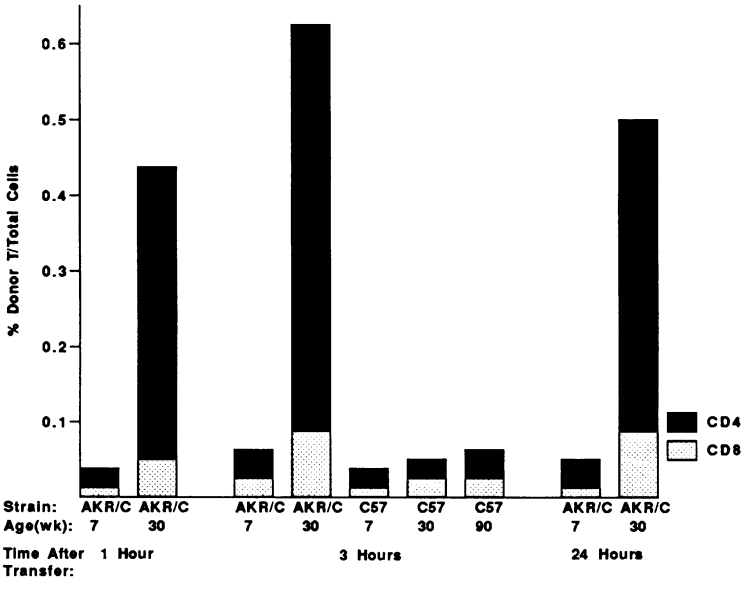

Figure 9. *CD4* versus *CD8 phenotypes of peripheral T cells that traffic to thymus. Host mice received 5 × 10^7 LN T cells from Thy-1 congenic donor mice. The transferred AKR donor T cells were 76% $CD4^+$, 24% $CD8^+$, while the C57BL/Ka donor T cells were 59% $CD4^+$, 41% $CD8^+$.*

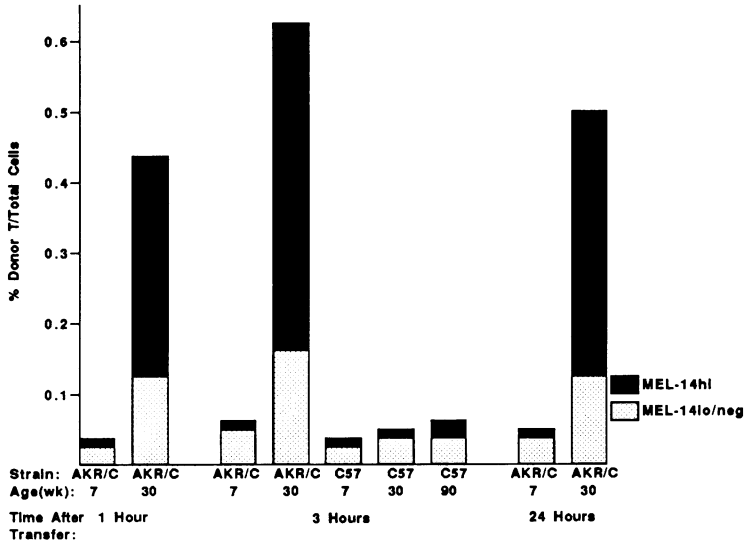

Figure 10. *Expression of MEL-14 antigen by peripheral T cells that traffic to thymus. The transferred AKR donor T cells were 72% MEL-14^{hi} while the C57BL/Ka donor T cells were 76% MEL-14^{hi}. The majority of T cells that migrated to AKR hyperplastic thymus were MEL-14^{hi}. In contrast, the minority of T cells that trafficked to histologically normal thymuses of young AKR or various age BALB/c mice were MEL-14^{hi}.*

circulate to the thymus are relatively enriched for MEL-14$^{lo/neg}$ cells.[9,10] We find here that while very young AKR mice demonstrate this same phenomenon, as the traffic of peripheral cells into the thymus increases with age in the hyperplastic, preneoplastic thymus, the immigrants express a level of MEL-14 comparable to that of normal peripheral lymphocytes. This finding suggests that at least one of the homing mechanisms functional in the hyperplastic thymus may be the same as in peripheral lymph nodes.

Immigrants derived from the periphery may contribute to some of the phenotypic abnormalities described in preleukemic mouse thymuses. Kawashima et al[5] found that 6-month-old AKR thymocytes as a population express an increased level of H-2 and decreased levels of Thy-1 and CD8 (Ly-2). This phenotype is consistent with that of peripheral T cells. While they found too many thymocytes to be affected to be accounted for entirely by recirculating cells, peripheral immigrants must contribute a significant fraction of cells bearing this phenotype. Zielinski et al[7] found increasing numbers of AKR thymic cells to express I-A with increasing age. When corrected for adherent cells, they report between 3% and 4% I-A$^+$ from 5 to 24 weeks and 13% for mice aged 28 to 40 weeks. We find B cells, which express high levels of I-A, to comprise between 1% to 2.5% and 3.5% to 4.5% of thymic cells in those two age ranges. This indicates that a sizable component of this observed phenotypic change is contributed by B cells and that it is not entirely due to a phenotypic change in classical thymocytes. Peripheral-derived T cells also may contribute to this phenomenon, although we have not observed I-A expression on recent peripheral T-cell immigrants. Newcomb et al[6] noted increased numbers of MEL-14^{hi} cells during the preneoplastic phase of carcinogen-treated C57 mice. Although this model may not be identical to the AKR mouse, our findings suggest that many such cells may be peripheral-derived B and T cells.

Thymuses of normal mice exhibit characteristics of peripheral lymphoid organs: mature T cells,[9] mature B cells,[10] antigen-presenting cells with access to circulating antigens[31] and participation in the recirculation pathway of peripheral lymphocytes.[9,10] These features suggest the ability of the thymus to form an organized peripheral lymphoid microenvironment, although in normal mice no such organization is apparent histologically and these elements are present at very low levels. The hyperplastic AKR thymus demonstrates that this potential can be realized, with increased numbers of T and B cells, recirculation features similar to peripheral lymphoid organs, and the formation of secondary structures (B-cell follicles with adjacent mature T-cell zones). These changes might be the passive result of a breakdown in a normal thymic barrier to lymphocyte recirculation, although our demonstration here of selective migration of various lymphocyte subsets to hyperplastic thymus makes this possibility unlikely. Alternatively these changes might be an active response to an antigenic stimulus in the AKR thymus. In this regard, we note that the first evidence of peripheral lymphoid influx is at 4 and 19 weeks of age in AKR/J and AKR/C mice, respectively. This is close to the timing of the first intrathymic appearance of the recombinant retrovirus at 6 and 10 weeks in these two strains.[4] Similar microenvironmental changes have been described in the thymuses of autoimmune (NZB × SJL)F1 mice[11] and in the thymuses of patients with myasthenia gravis.[32] In the latter condition, thymic B cells have been shown to produce autoantibodies reactive with thymic components.[33] We do not find evidence, however, in the AKR thymus for activation of immigrant lymphocytes. Peripheral derived T cells lack the interleukin-2 receptor (Tac) and the transferrin receptor and are

I-A negative and thymic B cells are largely IgM and D positive. Although germinal centers have been described in the AKR thymus,[1] we have observed them only rarely. Based on studies of the preleukemic mouse spleen, Lee at al[34] have proposed that a cellular immune response is required for murine retroviral leukemogenesis. We find that while the necessary components for such a response are present in the thymus, there is no evidence for their activation.

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