Development of Lymphoma in the Thymus of AKR Mice Treated with the Lymphomagenic Virus SL 3-3

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ABSTRACT

A chronological study of the individual thymic lobes of young AKR mice after neonatal inoculation of the oncogenic AKR retrovirus SL 3-3 was performed. 100% of mice treated in this manner develop lymphoma between 60 and 100 days of age. A search for early lymphoma cells in individual thymi was carried out by isolating the thymocytes successively in syngeneic and intrathymically in syngeneic and semisyngeneic recipients. Tumor progression was observed in animals between 48 and 60 days of age. These animals have: (a) normal weight lobes, in which no lymphomas cells could be detected, (b) thymus-dependent lymphoma cells, in one or both normal weight lobes; (c) thymus-independent lymphoma cells, found in lobes of normal weight as well as in thymi enlarged by lymphoma cells. Thymocyte characteristics of virus-treated animals of 21 to 63 days of age were compared with those of age-matched controls. Beginning at 28 days a concordant, progressive with time, increase of thymocyte surface staining for the viral envelope glycoprotein gp70 was seen in all lobes from virus-treated animals. Evaluation of cell surface markers by two-color fluorescence with antibodies to CD4 and CD8 showed that after 50 days of age, thymic lobes with and without lymphomas had nonspecific, but marked, alterations of the typical thymocyte surface marker pattern. No characteristic CD4, CD8 surface phenotype was found in primary lymphomas.

Using probes for the T-cell receptor Jg2 gene segments and the Akv ecotropic virus gp70 envelope genes, oligoclonality in Jg2 rearrangements and clonality using the Akv env genes was demonstrated in thymi with the thymus-dependent phenotype. In lymphomas T-cell receptor ß gene probes showed either oligoclonality or clonality. Clonal virus integrations were found in these lymphomas.

These experiments suggest the following series of events in virus-accelerated AKR lymphomagenesis. First, lymphoma cells arise which are initially thymus-dependent and can appear in one or simultaneously in both thymic lobes. These progress to become thymus-independent, fully autonomous, tumor cells. Thymocytes close to or at the time of the initial transformation event show a marked disorder of differentiation defined by the alterations in the CD4, CD8 surface phenotype distribution.

INTRODUCTION

AKR mice develop thymic lymphoma after 6 months of age with high frequency. This has been shown to be the result of the appearance of an oncogenic retrovirus with thymotropic properties in the thymus of these animals at 4-5 months of age (1). The appearance of oncogenic retrovirus followed by the development of lymphoma is random in the "natural" state of the AKR mouse. Oncogenic retroviruses from lymphomas of AKR mice, e.g., SL 3-3 (ecotropic) and MCF 247 (tropic) have been isolated and characterized at the molecular level (2, 3). They accelerate lymphoma development when inoculated into young AKR mice and induce lymphomas when inoculated into neonatal mice of other strains (4). Neonatal inoculation of these oncogenic retroviruses provides a group of animals for study in which the malignant disease occurs in a shorter time period than spontaneous disease and its time of development is clustered. Intrathymic events that precede lymphomagenesis in this model have been studied but have not been completely defined (5, 6).

The oncogenic retroviruses isolated from AKR lymphomas do not have oncogenes in their genome (7). In vitro transformation by these viruses has not been demonstrated. The latency to lymphoma development after their inoculation is more prolonged than that occurring after inoculation of retroviruses which contain oncogenes. Since it has been shown that the LTR of the SL 3-3 AKR retroviral genome confers its thymotropic and oncogenic properties (8), the question arises as to whether the malignant transformation of thymocytes, consistently resulting from infection of murine hosts with SL 3-3 virus, is the result of insertional mutagenesis caused by a specific viral integration and/or if it occurs following a viral-induced perturbation of thymic lymphopoiesis in the target organ. In order to gain further insight into the mechanisms of lymphomagenesis in this model system, we chose to study individual thymi (thymic lobes) at intervals after neonatal inoculation of SL 3-3, an ecotropic retrovirus isolated from a lymphoma cell line of AKR mice (7). In AKR mice, this virus causes thymic lymphoma with similar incidence and shorter latency than the polytropic recombinant viruses.

We are able to show in the studies reported here that malignant transformation is regularly preceded by a spread of virus infection in thymocytes beginning at 4 weeks after inoculation. This occurs in a thymus which continues to produce normal appearing, proliferating T-cells which are responsive to activation signals. Lymphomas can occur simultaneously in single thymic lobes and progress from a thymic-dependent to a thymic-independent phase. Thymi 53-60 days postvirus inoculation, with or without transplanted lymphoma cells, show a marked disregulation of surface phenotypic characteristics.

MATERIALS AND METHODS

Materials

Con A1 (C-7275) was from Sigma Chemical Co. and was diluted from a 1 mg/ml sterile-filtered stock solution in PBS. PMA (P-8139) was from Sigma and was diluted to make a stock solution of 10 μg/ml in 70% ethanol (NET-027E) was from New England Nuclear, Boston, MA. gp70 was used as a supernatant from hybridoma 24-8 (reactive with an epitope shared by the Akv and SL 3-3 env protein) provided by Drs.

1 The abbreviations used are: Con A, concanavalin A; PBS, phosphate buffered saline; PMA, phorbol 12-myristate 13-acetate; FITC, fluorescein isothiocyanate; PFCS, fetal calf serum; PBN, PBS + 0.2% bovine serum albumin + 0.2% Na3VO4; IL-2, interleukin 2; TD, thymus-dependent; TCR, T-cell receptor; PFTU, plaque-forming units.

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3 The abbreviations used are: Con A, concanavalin A; PBS, phosphate buffered saline; PMA, phorbol 12-myristate 13-acetate; FITC, fluorescein isothiocyanate; PFCS, fetal calf serum; PBN, PBS + 0.2% bovine serum albumin + 0.2% Na3VO4; IL-2, interleukin 2; TD, thymus-dependent; TCR, T-cell receptor; PFTU, plaque-forming units.
Dr. Miles Cloyd of the University of Texas Medical Branch at Galveston. It is specific for the envelope glycoprotein of these viruses. FITC-Goat $\alpha$-mouse IgG, affinity purified (1611-0081) was from Becton Dickinson Immuno-cytometry Systems, Mountain View, CA. Nonspecific controls, MSIGa-FITC and MSIGa-RD1 were from Coulter Immunology, Hialeah, FL. Media used were RPMI + 10% FCS (R10F), RPMI + 0.2% bovine serum albumin (RB/S) and PBN. AKR/J mice were bred in our facility, (AKR/JxDBA2JF), (ADF,) were from The Jackson Laboratory, Bar Harbor, ME.

Methods

Virus Inoculation. Lymphoma was induced by inoculating i.p. 3-4-day-old AKR pups with 0.1 ml of SL 3-3 virus-containing supernatant (10^7 PFU/ml). The supernatant was obtained from NIH 3T3 cells infected with the molecularly cloned virus (2).

IL-2-containing Supernatant. Supernatant was obtained by stimulating a culture of EL4.E1 cells at a concentration of 1 h/ml with 20 ng/ml PMA for 24 h. The supernatant was extracted for 5 min with 1 pel of dextran-coated charcoal per milliliter. After centrifuging down the charcoal, the supernatant was 0.2-$\mu$m filter sterilized.

Preparation of Thymocytes. The two lobes of the thymus were weighed separately on stainless-steel screens in tared dishes and extracted with RB/S medium, spun down and washed in RB/S, and suspended in 2 ml RB/S. The cells were counted in a hemocytometer and diluted or concentrated to 5 x 10^6/ml in R10F, 50 x 10^6/ml in R/B/S, and 200 x 10^6 in R/B/S.

Morphology of Thymocytes. This was assessed by counting 200 cells on Wright’s stained cytocentrifuge preparations. Small cells were 10 $\mu$m or less in diameter with a dense nucleus and scant cytoplasm. Large cells were 10-20 $\mu$m in diameter with a more open chromatin pattern in the nucleus and abundant cytoplasm.

Spontaneous Thymic Uptake. Freshly extracted thymocytes were immediately diluted 1:10 and 1:100 in R10F. 100 $\mu$l of these dilutions were incubated in duplicate with 2 $\mu$g of antibody or 1% IL-2 supernatant. The warm medium was mixed into the melted agarose and immediately half of it was dispensed in 0.5-$\mu$l aliquots into wells of a 24-well plate. The rest of the agarose medium was kept at 4°C. 120 min later the plate was stopped with 5 $\mu$l of 20% cold thymidine and the cells were collected on fiber filters in a Mini-MASH apparatus. The filters were oven-dried and counted in a scintillation vial. Results are expressed as CPM/1000 cells.

Colony-forming Assays. 50 mg of agarose and 1 ml of PBS were autoclaved in a 15-ml polypropylene tube which was subsequently kept in a 45°C water bath. Another tube containing 9 ml of medium was also heated to 45°C in the bath. The basic medium was RPMI with 10% FCS and contained either 2 $\mu$g/ml Con A and/or 1% IL-2 supernatant. The warm medium was mixed into the melted agarose and immediately half of it was dispensed in 0.5-$\mu$l aliquots into wells of a 24-well plate. The rest of the agarose medium was kept at 4°C. The plate was chilled for 15 min in the refrigerator. Meanwhile 0.2 x 10^8 thymocytes in 50 $\mu$l were dispensed into small tubes. One ml of agarose medium was added to a tube of thymocytes and immediately dispensed into two chilled wells of agarose. This agarose layer set in about 5 min at room temperature. The plates were incubated at 37°C in 5% CO_2 for 7 days and scored for colonies using a dissecting microscope. All colonies contained 100-1000 cells.

Immunofluorescence and Flow Cytometry to Test for CD4 and CD8 Expression. 0.5 x 10^6 thymocytes were incubated for 30 min at 0°C with a mixture of 0.5 $\mu$g of the two monoclonal antibodies in 25 $\mu$l of PBN. The cells were washed by centrifugation through a layer of serum and further with PBN. They were suspended in 0.5 ml of 1% formaldehyde in PBS. As a control, cells were stained with fluorescent isotope-match, dye-coupled IgGs that do not react with thymocytes. To test for gp70 expression, 0.5 x 10^6 thymocytes were incubated for 30 min at 0°C with 50 $\mu$l of 24-8 supernatant in V-well microtiter plate. The cells were washed three times with 0.1 ml of PBN. They were indirectly stained with 25 $\mu$l of FITC-Goat-anti mouse IgG at a 1:20 dilution under the same conditions. The cells were then suspended in 0.5 ml of 1% formaldehyde in PBS. The control for the primary antibody was fresh medium. Cells stained with monoclonal antibodies were analyzed for CD4, CD8, gp70, Thy-1.1 (HO-22-1) and 1.2 (HO-13-4) on a Coulter Instruments FACS.

Inoculation of Thymocytes. AKR hosts were lightly ether anesthetized, one side of their backs was shaved, and 5 x 10^6 cells in 0.1 ml was injected s.c. AKR× DBA₂ (ADF,) hosts were anesthetized with Nembutal (90 $\mu$g/g), their chest and neck was shaved, a 1-cm incision was made over the sternum, and a 0.5-cm cut was made from the sternal notch down. The exposed thymus was injected with 5 x 10^6 cells in 25 $\mu$l using a 500-$\mu$l Hamilton syringe with a stepper. The wound was closed with two clips. Our early results were similar in mice with and without pretreatment with 400 rads $\gamma$Co irradiation, therefore, in most of the experiments irradiation was omitted from the protocol. ADF, mice were used so that the origin of developing lymphomas could be determined. Donor-type lymphomas were Thy-1.1 positive Thy-1.2 negative.

Southern Blots. Genomic DNA was prepared from normal 50-day-old AKR thymus, liver and brain, the normal-appearing thymus of 50-day-old mice treated with SL 3-3 virus at 3 days of age and from primary thymic lymphomas according to (9). The DNA was digested with the restriction enzyme EcoRI, electrophoresed on 0.8% (w/v) agarose gels and transferred to nylon membranes (BioRad, Richmond, CA). It was hybridized with probes for the J$\beta_1$ and J$\beta_2$ gene segments of the TCR $\beta$ locus (10) and with a 400-base pair Xmal fragment that detects the env gene of the AKR ecotropic viruses (11). Since SL 3-3 virus does not have an internal EcoRI site, any new bands detected by Southern blot analysis larger than 8.2 kilobases indicate the presence of full-length viral genome.

RESULTS

A Search for Small Numbers of Lymphoma Cells in the Thymus from Virus-treated Animals. This study was designed to see when lymphoma cells first appeared in the thymic lobes of mice inoculated with the oncogenic SL 3-3 virus at birth. In order to do this, we had to be able to detect low numbers of lymphoma cells in the presence of normal thymocytes. Since no specific lymphoma cell marker is available, their presence must be detected by transplantation. Serial titrations were carried out to see how many lymphoma cells were needed to produce a transplanted lymphoma. A putative prelymphoma thymus was simulated by adding a known number of lymphoma cells to 10^6 normal thymocytes and inoculating the cells s.c. into 1-month-old syngeneic mice. As few as 10^2 lymphoma cells could give rise to s.c. tumors within 20-35 days. As expected, the incidence of lymphoma increased and latent period to tumor development decreased with increasing cell number (Fig. 1).

The lymphomagenic properties of the SL 3-3 virus used for these studies are shown in Fig. 2. Cells from thymic lobes from SL 3-3 virus-inoculated mice 21-56 days of age were tested for the presence of lymphoma cells. In 48 lobes of normal weight from 24 mice, no transplanted lymphomas developed after s.c. inoculation in syngeneic hosts. In order to test if there was a stage of lymphoma development where the thymic microenvironment was required, 14 mice, with normal sized thymus at 48 and 60 days of age, that had been treated with virus soon after birth were studied. The cells were inoculated intrathymically into ADF, mice and s.c. into AKR mice. Thymus cells from 10 animals produced lymphoma of donor origin after intrathymic inoculation but not after s.c. inoculation (TD, lymphoma). Cells from four thymus produced lymphoma of donor type after both routes of inoculation (TI, lymphoma). Individual lobes from thymi of nine animals which were of normal weight, i.e., <55 mg, were studied. Two of these mice, at 57 and 59 days of age, had no lymphoma cells demonstrated in either lobe. Three mice at 58 and 59 days of age, had TD lymphoma cells demonstrated in one lobe, in the other lobe no
TUMOR PROGRESSION IN AKR LYMPHOMA

Fig. 1. A test for the presence of small numbers of lymphoma cells in an inoculum. One-month-old AKR mice, 40 per group, were inoculated s.c. in a shaved area in the intrascapular region, with $10^5$ normal AKR thymocytes + the indicated number ($10^4$, $10^3$, $100$) of lymphoma cells. The animals were observed for development of tumor nodules at the injection site. The lymphoma cells were from SL 3-3 virus-accelerated lymphomas.

Fig. 2. Incidence of thymic lymphoma, as judged by massive enlargement of the thymus with lymph node enlargement and hepatosplenomegaly, in AKR mice inoculated i.p. 3-4 days of age with SL 3-3 virus $10^3$ PFU/ml. 79 mice were inoculated, 77 mice developed lymphoma by 96 days.

lymphoma cells were detected. Two mice at 59 and 60 days had TD cells demonstrated in both lobes. One animal at 57 days had TI lymphoma cells in both lobes. One animal of 60 days had TD cells in one lobe, no lymphoma cells in the other; two had TD cells in both lobes; one had TD cells in one lobe and TI cells in the other; one had TI cells in both lobes.

Other Characteristics of Thymocytes from Normal Weight Single Thymic Lobes of SL 3-3 Virus-inoculated Mice. Animals were divided in groups of four; one control and two to three virus injected. They were evaluated at 21, 28, 33, 37, 40-41 (five injected, two controls), 45, 49, and 56 days of age. The only remarkable difference prior to 40 days between experimental and control mice was, as has been described in previous studies (6, 12), in the thymocyte expression of the viral envelope glycoprotein (gp70). It was found to increase progressively on the surface of cells from both lobes in the SL 3-3 virus-treated group after 28 days (Fig. 3).

Studies using the CD4 and CD8 monoclonal antibodies revealed nearly identical and typical normal flow cytometry patterns in individual lobes from the 20 virus-inoculated animals and four controls of 21-37 days of age. These were: CD4$^+$ 7.3%, SD 2.5; CD4$^+$ 74%, SD 6.0; CD4$^+$ 11.2%, SD 2.5; CD8$^+$ 6.8%, SD 1.7. Between 40 and 56 days of age, four virus-treated animals with increased percentages (15-38%) of CD4$^+$ cells in one or both lobes were found. In the 17 virus-treated animals studied at 57-63 days of age, the flow cytometry patterns were abnormal in at least one lobe of each animal. In fact, there were only five of 34 lobes with typical flow cytometry patterns. Three were in lobes in which no lymphoma cells were found, one was in a lobe with thymus-dependent and the other with thymus-independent lymphoma cells. The changes in surface markers were remarkable and varied. Some showed increased percentages of CD4$^+$ cells, some increased CD4$^+$ or CD4$^+$ cells or both with a concomitant reduction of CD8$^+$ cells.

The thymic weights were slightly lower than controls at 45 and 49 days of age but cellularity, morphology of thymocytes, thymocyte colonies formed in the presence of Con A and IL-2

![Graph](image_url)
and proliferative capacity as measured by thymidine uptake revealed no significant differences from age-matched controls (Table 2).

**Surface Phenotype Characterization in Primary Lymphomas.**

The thymi from 24 primary SL 3-3 virus-accelerated lymphomas were studied for surface marker expression. In some instances, thymic colonies were measured and surface gp70 was evaluated. The remarkable finding was a heterogeneity of surface marker expression. In each tumor, there was a predominance of cells of one or sometimes two phenotypes. Eighteen lymphomas had a predominance >60% of a single marker: CD4-8- (one); CD4+8- (10); CD4-8+ (three); CD4+8- (four). The remaining six lymphomas showed a predominance of cells representing two phenotypes, i.e., CD4-8-/CD4-8+ (two); CD4-/CD4-8+ (three); CD4-/CD4+8 (one). In each lymphoma a minority of cells with the other phenotypes were always represented.

The lymphoma thymocytes were >95% positive for gp70. The colony assay showed some lymphomas with colony formation in the absence of added factors, some formed colonies in the presence of IL-2 or Con A alone and some, like the normal thymocytes, required both IL-2 and Con A for colony formation (data not shown).

**Studies of DNA from Prelymphoma and Lymphoma Thymi for TCR Gene Rearrangements and New Virus Integration.**

In these studies, we used three probes to study DNA from prelymphoma thymi and primary lymphomas induced by SL 3-3 virus. These were the probes for the TCR Jβ1 and Jβ2 gene segment clusters and the AKR virus env gene. All were hybridized to Southern blots of EcoRI-digested DNA. Three groups of animals were studied. 50-day-old mice treated with SL 3-3 virus at 3 days of age, 50-day-old age-matched controls (both of these groups had thymi of normal weight and appearance) and 60-70-day-old mice with thymic lymphomas. The cells from the normal-sized thymi of virus-treated mice were also inoculated intrathymically into ADF mice, and s.c. into AKR mice to test for the presence of either TD or TI lymphoma cells. In the five normal-sized thymi from virus-treated mice, three had TD lymphoma cells and in two no lymphoma cells were detected. Cells from the primary lymphomas produced s.c. tumors with a latency of 25 days.

The EcoRI digests of DNA from thymi of the two virus-treated animals in which no lymphoma cells could be detected showed polyclonal TCR β gene rearrangements and the absence of the 10-kilobase germine Jβ1 band (data not shown). This DNA showed polyclonal TCR β gene rearrangements and the presence of a small amount of the 2-kilobase germ line DNA when hybridized with a Jβ2 gene segment probe (Fig. 4, lanes 4 and 6). With regard to these properties, this DNA was very similar to normal thymus DNA (Fig. 4, lanes 1, 9, and 10). The pattern of hybridization of control thymus DNA and DNA from the two thymi with no lymphoma cells with the gp70 probe (Fig. 5, lanes 1, 4, and 6) was identical to that in nonlymphoid tissue from AKR mice (data not shown). In contrast, DNA from one thymus with TD cells showed oligoclonal Jβ gene segment rearrangement (Fig. 4, lane 2) and new virus integrations (Fig. 5, lane 2). A second TD thymus DNA showed a predominant Jβ2 gene segment rearrangement (Fig. 4, lane 3). This DNA was very similar to that in nonlymphoid tissue from AKR mice (data not shown). In contrast, DNA from one thymus with TD cells showed oligoclonal Jβ gene segment rearrangement (Fig. 4, lane 2) and new virus integrations (Fig. 5, lane 2). A second TD thymus DNA showed a predominant Jβ2 gene segment rearrangement (Fig. 4, lane 3) and new virus integration (Fig. 5, lane 3) and in DNA from the thymus of the third mouse with thymus-dependent lymphoma cells, Jβ2 rearrangements were polyclonal (Fig. 4, lane 5) but new virus integration sites were present (Fig. 5, lane 5). DNA from primary lymphomas of the thymus showed some with clonal or oligoclonal Jβ1 or Jβ2 rearrangements. Each rearrangement was unique for that lymphoma. New virus integrations were demonstrated in all tumor DNAs.*

**DISCUSSION**

The virus-accelerated development of thymic lymphoma in the AKR mouse provides a model for looking at factors in the intact animal which preclude the malignant process and for following the events leading to progression of the tumor. In a relatively short period of time 100% of virus-inoculated animals develop a malignant disease of thymocytes that has

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* Manuscript in preparation.
identical gross and microscopic morphology. This model removes factors which cause problems when studying the development of the spontaneous T cell lymphoma in AKR mice. These are: (a) the confusion of age-related versus virus-related changes in the "preleukemic" thymus since spontaneous disease can occur at any time between 7 and 15 months of age. During this period the thymus undergoes an age-related involution; (b) the spontaneous disease, from 6 to 14 months of age, occurs in a random fashion following the appearance of oncogenic retroviruses (13). Therefore, at any one point in time, an animal could be days or months away from oncogenic virus production and subsequent lymphoma development; (c) there can be no control animals when studying preleukemic changes prior to spontaneous disease.

Studies of the virus-accelerated lymphoma of AKR mice using intrathymic injection of adults with a dualtropic virus-related oncogenic retrovirus MCF 69-1 have been reported (6). In this model animals develop lymphoma 60–100 days post inoculation, i.e., at 105 to 145 days of age. Spread of virus infection throughout the thymocyte population was noted and, as in the current study, it preceded specific changes in thymocytes. No virus infection spread of virus infection was noted and, as in the current study, it preceded specific changes in thymocytes. No virus infection

Fig. 5. Southern blot analysis using EcoRI-digested DNA and AKR viral env probe. Lane 1 is normal thymus. Lanes 2–5 are as designated in Fig. 4. Lanes 2 and 5 show new virus integration sites. Lanes 1, 3, 4, and 6 show the EcoRI pattern of the endogenous ecotropic viruses of AKR mice. The molecular weight bands migrating below 10.8 kilobases can be removed by more stringent washing of the filter.

Zielinski et al. (14) studied phenotype expression in the course of development of AKR spontaneous lymphoma and determined that a shift to a mature thymocyte phenotype occurs during the leukemogenic process. This was determined by detection of increased La and H-2 antigens as lymphomas developed in the thymus.

The present study was designed to correlate the emergence of lymphoma cells with changes in thymocytes in AKR mice inoculated neonatally with SL 3-3 retrovirus. The data provide evidence for a two-step process of thymic lymphomagenesis. Thymic lobes were identified which were normal in weight and in which only thymic-dependent lymphoma cells were found, i.e., these cells produced tumors when transplanted into the thymus but not when transplanted s.c. This, presumably, is the earliest detectable cell transformation event. These thymus-dependent lymphoma cells were found either in both lobes or in single lobes of individual mice suggesting that transformation can occur simultaneously in each thymus lobe. A progression of lymphomas to further autonomy was demonstrated by the finding of lymphoma cells, again in normal sized organs, which transplant by both intrathymic and s.c. routes. The unilateral nature of this second event was demonstrated by the finding of thymus-dependent lymphoma cells in one lobe and thymus-independent cells in the other lobe of a single thymus. We call the change from TD to TI phenotype progression because the final result, lymphoma, is of the TI phenotype and the predominant phenotype in the 50-day-old normal-sized thymus from the virus-treated mouse is TD (Table 1). The possibility exists that there are two sets of events occurring and that each phenotype is distinct. However, when TI cells are present, the dependent phenotype cannot be detected. The blot hybridization studies support idea of progression. Oligoclonal expansion of thymocytes was shown to occur at the stage where TD lymphoma cells are present, suggesting that at this early stage clonal cell populations are in sufficient numbers to be detected by this methodology. Clonality was demonstrated using the TCR and viral probes when the thymus was enlarged and replaced by tumor cells. Thus, oligoclonal proliferation as shown by J/6 gene segment rearrangements and clonal expansion as shown by new viral integrations occur at an early stage (TD) in lymphomagenesis.

It is also of interest that the TI cells, injected intrathymically produced lymphomas in recipients within 3–4 weeks. On the other hand, the TD lymphoma cells have a prolonged latency after intrathymic inoculation. Thus, we show that the TD cells and their progeny can remain in the thymus for a prolonged time after intrathymic transplantation. The intrathymic residence of normal thymocytes has been described to be less than 3 weeks (15). This suggests that the TD lymphoma cells, in contrast to normal immature thymocytes, which have been shown to differentiate intrathymically (15), have acquired a capacity for self renewal within the thymus. It should be noted that long-latency, thymus-dependent tumor cells have been demonstrated in mice after split-dose irradiation or inoculation of radiation leukemia virus (16, 17).

As shown by the studies reported here, retroviral infection, measured by surface gp70 on thymocytes, involves increasing numbers of cells beginning at 28 days of age. We have previously shown that biologically active lymphomagenic virus is present in the thymus of AKR mice as early as 1 week after SL 3-3 virus treatment (18) 3 weeks before the demonstrated increase in thymocyte gp70. This suggests that the thymic stromal cells which are permissive for this virus (19) may be the initial target of infection.

In virus-inoculated animals after 57 days of age, there was a marked and varied deregulation of the surface phenotype profile as measured by CD4 and CD8 two-color fluorescence. This occurred in thymi without lymphoma cells as well as normal-sized organs at both stages of tumor progression and after thymic enlargement by lymphoma cells. There is no consistent surface phenotype represented in the lymphomas suggesting...
that all stages of thymocyte maturation are susceptible to transformation and/or that erratic patterns of differentiation occur before and after the onset of transformation. This change appears to be directly related to virus infection as it spreads to include more thymocytes and is closely related to the onset of malignant change.

On the basis of these and past observations, we postulate that the series of events leading to autonomous metastatic lymphoma after neonatal oncogenic retrovirus infection are as follows: shortly after i.p. inoculation the infectious virus enters the thymus first establishing infection in thymic stroma. After 4 weeks, the thymocytes become progressively infected with retrovirus detected by appearance of the viral envelope glycoprotein on their surface. At approximately 50 days of age, cells appear which can give rise to lymphoma but are dependent on the thymic environment for proliferation. These are found in a single lobe or simultaneously in both lobes. This TD phenotype follows: shortly after i.p. inoculation the infectious virus enters the thymus first establishing infection in thymic stroma. After 4 weeks, the thymocytes become progressively infected with retrovirus detected by appearance of the viral envelope glycoprotein on their surface. At approximately 50 days of age, cells appear which can give rise to lymphoma but are dependent on the thymic environment for proliferation. These are found in a single lobe or simultaneously in both lobes. This TD phenotype due to virus integration in or near genes related to T-cell activation. The lymphoma cells at each stage of tumor progression respond to differentiation signals in a variable manner. The result of this process is production of the typical thymic lymphoma with varied surface phenotypic characteristics.

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