Radiation Leukemia Virus-Induced Thymic Lymphomas Express a Restricted Repertoire of T-Cell Receptor Vβ Gene Products

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We have investigated the phenotypic changes that take place during the process of neoplastic transformation in the thymocytes of C57BL/Ka mice infected by the radiation leukemia virus (RadLV). By the combined use of antibodies against the envelope glycoprotein gp70 of RadLV, the transformation-associated cell surface marker 1C11, and the CD3–T-cell receptor (TCR) complex, we found that in the RadLV-infected thymus, the earliest expression of viral gp70 is in 1C11^{hi} cells; a small but significant percentage of these cells also express CD3. A first wave of viral replication, manifested by the expression of high levels of gp70 in thymocytes (over 70% positive), reaches a peak at 2 weeks; during this period, no significant changes are observed in the expression of 1C11 or CD3. The population of gp70$^+$ cells is drastically reduced at 3 to 4 weeks after infection. However, a second cohort of gp70$^+$ cells appears after 4 weeks, and these cells express high levels of 1C11 and TCR determinants as well. RadLV-induced lymphomas differ from normal thymocytes in their CD4 CD8 phenotype, with domination by one or more subsets. Characterization of TCR gene rearrangements in RadLV-induced lymphomas shows that most of these tumors are clonal or oligoclonal with respect to the Jβ2 TCR gene, while the Jβ1 TCR gene is rearranged in a minority (4 of 11) of lymphomas. TCR Vβ repertoire analysis of 12 tumors reveals that 6 (50%) express exclusively the Vβ6 gene product, 2 (17%) are Vβ5$^+$, and 1 (8%) each are Vβ8$^+$ and Vβ9$^+$. In normal C57BL/Ka mice, Vβ6 is expressed on 12%, Vβ5 is expressed on 9%, Vβ8 is expressed on 22%, and Vβ9 is expressed on 4% of TCR^{hi} thymocytes. Thus, it appears that RadLV-induced thymic lymphomas are not randomly selected with respect to expressed TCR Vβ type.

Exposure to ionizing radiation is known to induce lymphoid malignancy in humans; the incidence of acute lymphoblastic leukemia in particular has been shown to correlate with radiation dose (34). To facilitate investigation of the basic mechanisms by which radiation induces leukemia, Kaplan and colleagues developed a murine model (21, 22) which they used to define the parameters for T-cell transformation. Whole-body irradiation, administered in fractionated doses, was found to induce a high incidence (>90%) of thymus-dependent lymphomas in C57BL/Ka mice (22). A thymotropic and lymphomagenic retrovirus, the radiation leukemia virus (RadLV), was recovered from cell extracts of some of these lymphomas (29, 30). After serial in vivo passage, RadLV-induced thymic lymphomas were found to be similar in frequency, clinical aspects, and latency to those produced by radiation. Several lymphoma cell lines from RadLV-induced tumors were established in culture (27); one such cell line, BL/VL3, was used to molecularly clone the RadLV proviral genome, and its complete nucleotide sequence has been determined (33). It is believed that RadLV and other thymotropic and leukemogenic murine leukemia viruses (MuLVs) have evolved as a result of recombinational events between ecotropic and xenotropic endogenous retroviral sequences present in the mouse genome (4).

Structural analysis of the viral genome revealed the presence of tandem repeats in the long terminal repeat region, similar to those observed in other leukemogenic retroviruses (8, 24, 26). The repeats are believed to be responsible, in part, for the tissue tropism and leukemogenicity of these viruses (8, 26). Studies of recombinant nonleukemogenic and leukemogenic mink cell focus-forming viruses have shown that, in addition to the long terminal repeat, the envelope gene product also determines the leukemogenic potential of MuLVs (9, 17). Although elucidation of the molecular and structural properties of RadLV has advanced our understanding of the virus, the mechanism by which RadLV causes T-cell transformation is still unclear. The receptor-mediated leukemogenesis hypothesis (47) proposes a possible step in the pathway for retrovirus-induced neoplastic transformation. It proposes that a crucial step is the binding of MuLV to a cell surface mitogen receptor such as the clonotypic T-cell receptor (TCR) on target T cells, presumably inducing mitogenic signal transduction. Subsequently, O'Neill et al. demonstrated that in the T-cell lymphoma line C6VL, binding of the retrovirus may be blocked by the TCR clonotypic antibody and that, as a result, proliferation of the leukemic cells is inhibited (38). Other steps in the process have also been proposed, including activation of proto-oncogenes by nearby integration of retroviral genes with enhancer activity (16, 26).

The target cell type and the events occurring in the course of lymphomagenesis within the infected thymus have yet to be fully defined. The availability of monoclonal antibodies against T-cell differentiation antigens (3, 25) and the transformation-associated antigen 1C11 (28, 41) and of hybridization probes to

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TCR genes (7) have made possible an analysis of surface marker phenotypes of RadLV-infected, preleukemic, and/or leukemic thymocytes. Using a monoclonal antibody against the gp70 glycoprotein of RadLV, we present evidence that the earliest cell type in the RadLV-infected thymus to express viral gp70 is 1C11^{hi} and that a subset of these cells express high levels of the TCR-CD3 complex. The first wave of thymic viral replication, marked by a rising percentage of gp70-positive thymocytes of several phenotypic classes, reaches a peak at 2 to 3 weeks after infection. A transient drop in this population occurs at 3 to 4 weeks, followed by a permanent rise in gp70$^+$ cells that mainly express high levels of both 1C11 and CD3. The ensuing RadLV-induced thymic lymphomas exhibit phenotypic profiles which are not detectable in the normal thymus. TCR gene rearrangement data obtained by using probes to Jβ1 and Jβ2 sequences show that the majority of these tumors are clonal or oligoclonal, and each lymphoma expresses only one TCR-Vβ. The Vβ-bearing subsets expressed by these lymphomas differ dramatically from the Vβ profile of normal C57BL thymocytes, suggesting that the Vβ component of the TCR in these tumors is not randomly selected.

MATERIALS AND METHODS

Mice and tumors. C57BL/Ka mice were obtained from the animal facilities of the Department of Comparative Medicine at Stanford University. The preparation of RadLV and its induction of thymic lymphomas were described previously (27). The virus was inoculated intrathymically in 5- to 6-week-old mice. At various intervals thereafter (from 3 days until the development of lymphomas), groups of three to five mice were sacrificed, and individual thymus cell suspensions were prepared for analysis. The phenotypic plots shown in the text figures represent typical patterns for the individual experimental groups.

Cell lines and antibodies. The T-cell lymphoma lines used in these studies were BL/RL12, a MuLV-free cell line derived from a radiation-induced C57BL/Ka thymoma, and BL/VL3, a MuLV-producing cell line derived from a RadLV-induced thymoma (27). The anti-RadLV gp70 hybridoma clone JM11 was generated by fusing splenocytes from a rat immunized against the BL/VL3 cell line with the mouse myeloma cell line XAg63. All experiments described here were carried out with the hybridoma culture supernatant. Sources, specificities, and fluorochrome modifications of monoclonal antibodies specific for CD3, CD4, CD8, 1C11, and $\alpha\beta$TCR have been described elsewhere (41, 42). Specificities and sources of other antibodies were as follows: anti-CD3, clone 145-2C11 (Boehringer Mannheim, Indianapolis, Ind.); phycoerythrin-conjugated anti-CD4, clone GK1.5 (Becton Dickinson, Mountain View, Calif.); goat anti-rat Ig conjugated to Texas red (TR) or fluorescein isothiocyanate (FITC) (Caltag Laboratories, South San Francisco, Calif.); and goat anti-mouse Ig conjugated to FITC, avidin-allophycocyanin (APC), and avidin-TR (Caltag). The following anti-Vβ TCR antibodies were used: B20.6 (Vβ2 [12]), KJ-25 (Vβ3 [39]), MR9-4 (Vβ5 [40]), RR4-7 (Vβ6 [20]), R-310 (Vβ7 [37]), F23.1 (Vβ8.1-8.3 [43]), KJ-16 (Vβ8.1-8.2 [23]), MR10-2 (Vβ9 [46]), RR3-15 (Vβ11 [5]), and MR12-3 (Vβ13 [48]). The antibodies against Vβ -3, -5, -6, -9, -11, and -13 were generous gifts from O. Kanagawa (Washington University, St. Louis, Mo.). Anti-murine $\alpha\beta$-TCR heterodimer antibody was kindly provided by R. Kubo (National Jewish Hospital, Denver, Colo.).

Flow cytometric analysis. The immunofluorescence staining procedure for one-, two-, three-, and four-color assays has been described in detail elsewhere (41, 42). The fluorescence of stained normal or RadLV-infected thymocytes and of thymic lymphomas was analyzed on a highly modified dual-laser FACS IV (Becton Dickinson) with four-decade logarithmic amplifiers as described previously (41). Dead cells were gated out with 1 μg of propidium iodide per ml except for four-color analysis, in which case the scatter-gating method was used and the excitation wave length of the dye laser was raised from 598 to 605 nm. Fluorescence profiles were analyzed by using FACS/DESK software programs to generate either histograms or two-parameter probability (5%) contour plots in which both x and y axes represent fluorescence intensity on a logarithmic scale. In describing the results, we use the terms "hi" and "lo" as relative indicators of staining intensity; hi cells usually stain at least 5- to 10-fold brighter than lo cells, which stain significantly but only slightly over background.

Categorization of normal thymic subsets by CD3, CD4, and CD8 staining. Adoptive transfer of fluorescence-activating cell sorter (FACS)-selected thymic lymphocytes into the thymuses of unirradiated Thy-1 congenic mice has revealed the following developmental pathway in C57BL/Ka mice (3, 13, 14): CD3$^-$ CD4$^-$ CD8$^-$→CD3$^-$ CD4$^-$ CD8$^+$→CD3^{lo} CD4$^+$ CD8$^+$ blast cells. CD3^{lo} CD4$^+$ CD8$^+$ blast cells have three developmental options: the majority of these cells rapidly becomes small CD3^{lo} CD4$^+$ CD8$^+$ cells that then undergo programmed cell death. About 10% enter the CD4 pathway, transiting through a CD3^{med} CD4$^+$ CD8^{lo} intermediate to become CD3^{hi} CD4$^+$ CD8$^-$ cells, while about 5% transit through a CD3^{med} CD4^{lo} CD8$^+$ intermediate to become CD3^{hi} CD4$^-$ CD8$^+$ cells (13, 14). We have designated the three progenitor classes (CD3$^-$ CD4$^-$ CD8$^-$, CD3$^-$ CD4$^-$ CD8$^+$ and CD3^{lo} CD4$^+$ CD8$^+$ blast cells) as immature cells, the end-stage small CD3^{lo} CD4$^+$ CD8$^+$ subset as nonmature cells, the CD3^{med} CD4$^+$ CD8^{lo} and CD3^{med} CD4^{lo} CD8$^+$ subsets as transitional intermediates, and the CD3^{hi} CD4$^+$ CD8$^-$ and CD3^{hi} CD4$^-$ CD8$^+$ subsets as mature thymocytes. The 1C11^{hi} fraction is normally limited to the immature subsets (41).

Immunoprecipitation and gel electrophoresis. Lactoperoxidase-catalyzed surface iodination of RadLV-induced thymic lymphoma cells and of lymphoma cell lines BL/VL3 and BL/RL12 was performed as previously described (41). For immunoprecipitation, 500 μl of monoclonal antibody JM11 (anti-RadLV gp70) was allowed to react with 50 μl of rabbit anti-rat Ig coupled to protein A-Sepharose beads (Sigma Chemical Co., St. Louis, Mo.). The beads were then incubated with ^{125}I-labeled cell lysates (500 μl) and washed five times with 0.5% Nonidet P40-containing lysis buffer, and the antigen was finally eluted from the beads with 50 μl of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) sample buffer. The immunoprecipitated antigen was run on SDS–12% polyacrylamide gels under reducing conditions. After drying, the gels were subjected to autoradiography using Kodak X-Omat AR films (Eastman Kodak, Rochester, N.Y.) and Dupont Cronex Lighting Plus intensifying screens (Du-Pont Co., Wilmington, Del.).

Preparation of DNA and Southern blot hybridization. High-molecular-weight DNA was isolated by proteinase K digestion followed by phenol-chloroform extraction. DNA was digested with the restriction enzyme EcoRI, and restriction fragments were electrophoretically separated on 0.8% agarose gels. The DNA was acid depurinated before denaturation and transferred onto a nylon membrane. The filters were hybridized with 2 × 10^6 cpm of random hexamer-labeled probes (10) per ml in a hybridization mixture containing 50% formamide, 1.5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt's solution, and denatured salmon sperm
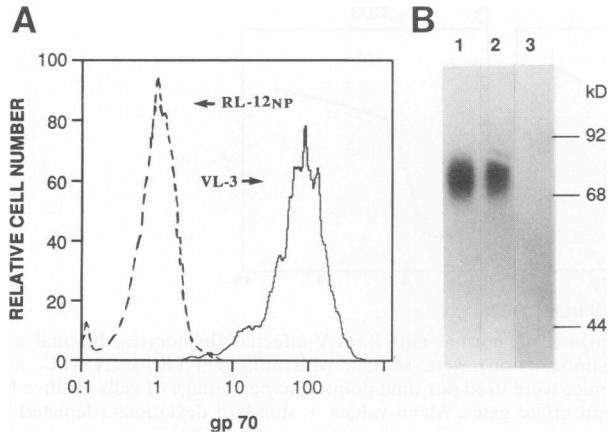
FIG. 1. Reactivity of the anti-RadLV gp70 monoclonal antibody JM11 with virus producer and nonproducer lymphomas. (A) Expression of gp70 on RadLV producer cell line BL/VL3 (solid line) and nonproducer cell line BL/RL12-NP (dashed line). In single-color fluorescence histograms, fluorescence intensity is presented (log scale) on the x axis versus cell number on the y axis. (B) Electrophoresis and autoradiographic visualization of the gp70 envelope antigen immunoprecipitated by antibody JM11 from ^{125}I-labeled cell lysates. Lanes: 1, BL/VL3; 2, primary lymphoma induced by RadLV; 3, BL/RL12-NP as a negative control. Samples were subjected to SDS-PAGE (10% gel) under reducing conditions. Apparent molecular masses were determined from the positions of size standards (Bio-Rad, Richmond, Calif.) run on the same gel.

DNA (100 mg/ml) at 42°C (11). The filters were washed with 0.1× SSC–0.1% SDS at 55°C and subjected to autoradiography as described above.

DNA probes. The probes used to detect TCR rearrangements were specific for the Jβ1 and Jβ2 gene segments of the TCR β locus (11). The probes were a kind gift from K. Ikuta of this laboratory.

RESULTS

Characterization of monoclonal antibody JM11, which recognizes viral gp70 protein. Anti-BL/VL3 hybridoma clones were characterized by immunofluorescence staining of the RadLV producer cell line BL/VL3 and subsequently by radio-immunoprecipitation analysis. FACS analysis, performed with five different hybridoma supernatants, showed reactivity to the BL/VL3 cells. A typical histogram obtained with clone JM11 is shown in Fig. 1A. The antibody exhibited strong reactivity with BL/VL3 but not BL/RL12 (RadLV-negative) lymphoma cells, indicating probable specificity for the viral antigen expressed on the cell surface. Next, the antigenic specificity of the antibody was determined by radioimmunoprecipitation, using iodinated cell lysates; the results are presented in Fig. 1B. The antibody immunoprecipitated a RadLV envelope glycoprotein of 70 kDa from both BL/VL3 cells (lane 1) and RadLV-induced primary lymphoma cells (lane 2) but failed to react with cell line BL/RL12 (lane 3).

Patterns of gp70, 1C11, and CD3 expression of RadLV-infected thymocytes. Thymocyte preparations were examined at different intervals after infection by multiparameter FACS analysis, using an anti-gp70 (JM11) antibody and antibodies to 1C11 and the TCR-CD3 complex. As shown previously, antibody to the heterodimeric antigen 1C11 recognizes a subset of normal thymic progenitors and transformed cells in irradiated mice (28, 40a, 41) as well as in Eμ–L-*myc* transgenic mice (35) during the preleukemic period. As early as 3 days after intrathymic RadLV inoculation, expression of viral gp70 could be detected on a small number (3 to 5%) of thymocytes. Almost all of the gp70$^+$ cells were 1C11^{hi} (Fig. 2). When examined for expression of the TCR-CD3 complex, a substantial percentage (20 to 40%) were also CD3$^+$. In the first 3 weeks after RadLV injection, the number of thymocytes expressing viral gp70 increased rapidly to over 70% of the population (Fig. 3A). Most of these cells did not express 1C11 (Fig. 3B) or CD3 (Fig. 3C). Between weeks 3 and 4 after infection, the percentage of gp70$^+$ thymocytes fell precipitously, only to rise again during the following 2 weeks. This
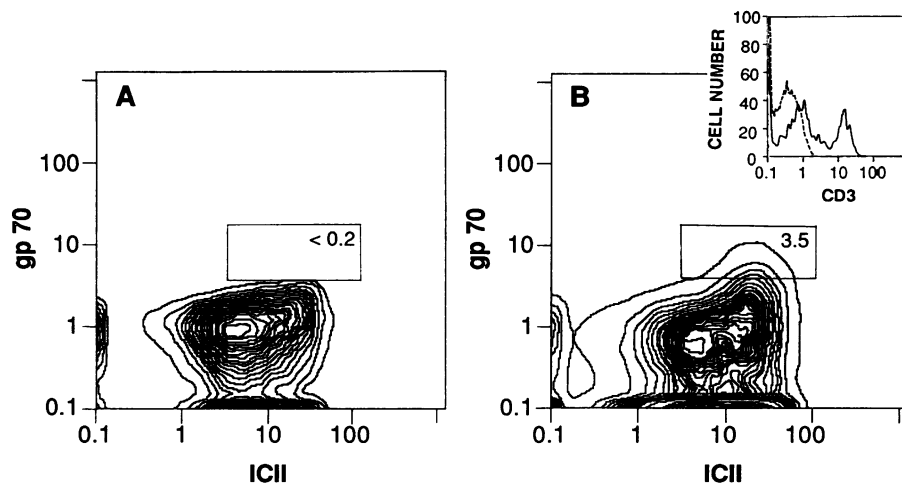

FIG. 2. Patterns of gp70 and 1C11 expression on normal (A) and RadLV-infected (day 4 after infection; B) thymocytes. Thymocytes were stained with anti-gp70 antibody followed by goat anti-rat Ig-TR. Next, the cells were stained with biotinylated 1C11–avidin-APC and anti-CD3-FITC. The rectangular area in each panel shows the percentage of cells that coexpress the gp70 and 1C11 antigens. The CD3 profile of the 3.5% of thymocytes which coexpress gp70 and 1C11 is shown in the insert in panel B (solid line). The broken line in the insert represents the background fluorescence.
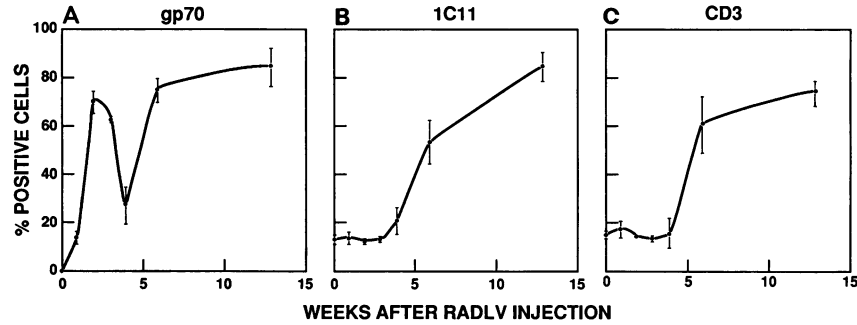
FIG. 3. Patterns of expression of gp70 (A), 1C11 (B), and CD3 (C) antigens on normal and RadLV-infected thymocytes. Normal and RadLV-infected thymocytes (obtained at 1, 2, 3, 4, 6, and 12 weeks postinoculation) were stained with anti-gp70-TR, 1C11-APC, and anti-CD3-FITC antibodies as described in Materials and Methods. Five to six mice were used per time point. The percentage of cells positive for each antigen was calculated from the corresponding histogram by setting appropriate gates. Mean values + standard deviations (depicted by vertical bars) are shown.

second wave of gp70 expression was permanent and was accompanied by parallel increases in cells expressing 1C11 and CD3 (Fig. 3). Cells bearing these three markers predominated thereafter, including the majority of cells in overt lymphomas.

CD4 CD8 phenotypes of RadLV-infected thymocytes and of primary lymphomas. No significant difference from normal cells was detected in the overall CD4 CD8 profiles of thymocytes from infected mice (data not shown) during the first 4 weeks after RadLV infection. The CD4 CD8 profiles of the resulting lymphomas were variable, and a representative set of data is presented in Fig. 4 along with the profile of normal thymocytes for comparison (Fig. 4A). Two of the lymphomas consisted predominantly of CD4$^+$ CD8$^-$ cells (Fig. 4B and C), while the third lymphoma was a mixture of CD4$^+$ CD8$^+$ and CD4$^-$ CD8$^+$ cells (Fig. 4D), a phenotype very frequently observed in primary radiation-induced lymphomas (40a). The composition of nine individual RadLV-induced lymphomas with respect to cells expressing 1C11, gp70, CD3, CD4, and CD8 is presented in Table 1. Unlike radiation-induced lymphomas, which are usually a mixture of CD4$^-$ CD8$^+$ and CD4$^+$ CD8$^+$ cells, the majority of RadLV-induced lymphomas were found to be heterogeneous with respect to CD4 CD8 phenotypes (Table 1). Four of eight lymphomas contained predominantly CD4$^+$ CD8$^-$ cells (>60%), and two consisted largely of the CD4$^+$ CD8$^+$ cell type (>60%). Of the remaining two tumors, one contained >70% CD4$^+$ CD8$^+$ cells, while the other one was a mixture of CD4$^+$ CD8$^+$ and CD4$^-$ CD8$^+$ cells. All lymphomas had also a small but significant percent-

age of CD4$^-$ CD8$^-$ cells. Various proportions of cells in the lymphomas, from <50 to >90%, expressed the gp70, 1C11, and CD3 markers (Table 1).

Since the majority of lymphomas coexpressed CD3 and gp70, 1 week after infection, we reanalyzed the profiles of the gp70$^+$ and gp70$^-$ thymocyte populations in infected mice with respect to CD3 expression subsets. In both populations, the CD3$^{-/lo}$ subsets showed a normal pattern of CD4 and CD8 expression, that of immature and nonmature CD4$^+$ CD8$^+$ thymocytes (data not shown). However, the CD3^{hi} subset was different: the infected population (gp70$^+$) consisted predominantly of CD4$^+$ CD8$^-$ and CD4$^+$ CD8$^+$ subsets and a few CD4$^-$ CD8$^+$ cells, whereas the CD4 CD8 phenotype of the uninfected population (gp70$^-$) was that of normal thymocytes (data not shown).

Restricted expression of Vβ TCR gene products in RadLV-induced lymphomas. The remarkably high TCR-CD3 antigen expression in RadLV-induced thymic lymphomas suggested that the expression of CD3 was nonrandom and that the Vα and Vβ subsets should be examined. The availability of antibodies against different Vβ elements made a study of the Vβ repertoire of the lymphomas possible. The expression of CD3 and the corresponding Vβ6 profile of a representative tumor are shown in Fig. 5. Over 90% of tumor cells expressed high levels of the CD3 antigen, and virtually all of this reactivity was attributable to their Vβ6 expression. Testing of a number of RadLV-induced lymphomas for Vβ usage resulted in the data presented in Table 2. Of the 12 tumors tested, 6
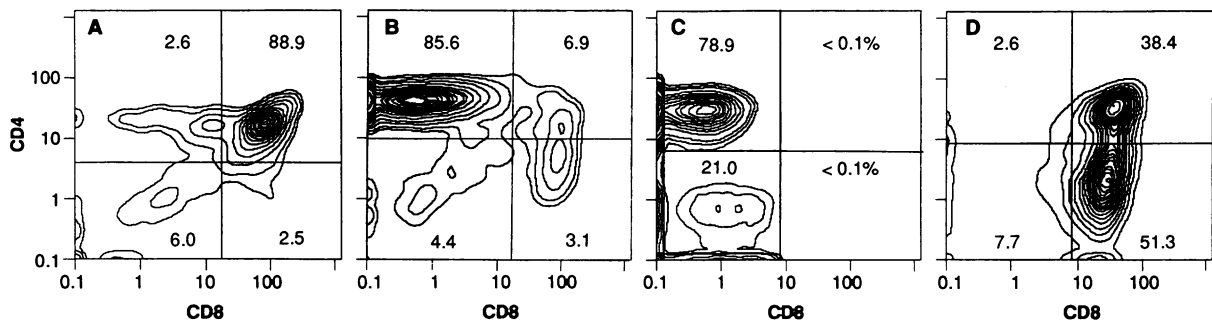

FIG. 4. CD4 versus CD8 profiles of normal adult thymocytes (A) and RadLV-induced lymphomas (B to D). Thymocytes and lymphoma cells were stained with phycoerythrin-conjugated anti-CD4 (Becton Dickinson) and biotinylated anti-CD8 followed by avidin-APC. Each profile is divided into four separate populations: CD4$^-$ CD8$^-$ (lower left), CD4$^+$ CD8$^-$ (upper left), CD4$^+$ CD8$^+$ (upper right), and CD4$^-$ CD8$^+$ (lower right). The staining pattern of normal thymocytes (A) served as a standard for determining the phenotypic subdivisions.

TABLE 1. Phenotypic characterization of RadLV-induced thymic lymphomas[a]

Thymus sample	% of cells expressing:			% with given CD4 CD8 phenotype			
	gp70	1C11^{hi}	CD3	CD4$^-$ CD8$^-$	CD4$^-$ CD8$^+$	CD4$^+$ CD8$^+$	CD4$^+$ CD8$^-$
Normal	<1.0	13.7	9.3	4.7	3.4	84.9	7.0
RadLV-induced lymphoma							
1	42.3	65.3	93.4	4.4	3.2	6.9	85.5
2	60.3	87.0	67.1	4.9	4.5	28.6	62.0
3	97.0	88.1	70.8	ND^b	ND	ND	ND
4	90.9	65.7	84.3	4.7	7.1	72.0	16.2
5	98.0	87.3	52.6	7.7	51.3	38.5	2.6
6	98.6	67.1	50.8	3.8	78.5	3.8	13.9
7	ND	97.5	71.0	8.7	9.5	61.2	20.6
8	95.9	ND	92.9	15.9	<1.0	<1.0	84.1
9	89.9	77.1	84.8	5.8	8.7	17.5	68.0

[a] Multiparameter FACS analysis was carried out as described previously (42).
[b] ND, not determined.

expressed exclusively Vβ6, 2 were Vβ5 positive, and one each were Vβ8 and Vβ9 positive. In normal adult C57BL/Ka mice, we found that 2.6% of total thymocytes express the Vβ8, 1.4% express the Vβ6, 1.1% express the Vβ5, and 0.5% express the Vβ9 element of the TCR heterodimer. Of the CD3^{hi} thymic subset in normal C57BL/Ka mice, the relative proportions are 22% Vβ8, 12% Vβ6, 9% Vβ5, and 4% Vβ9. The two remaining tumors did not bind any of the 10 antibodies used, although they tested positive for CD3 and the αβ TCR heterodimer (data not shown). The results suggest a restricted repertoire of TCR Vβ chain expression in RadLV-induced thymic lymphomas.

To determine any correlation between reappearance of virus-positive cells and clonal expansion of cells bearing a particular Vβ TCR, we analyzed four thymuses at 5 to 6 weeks after retrovirus injection. We found that in one of these mice, there was a considerable increase of cells expressing Vβ6 (44% of total thymocytes), and in another, there was an increase in Vβ5 (52% of total thymocytes) elements of the TCR. The remaining two mice did not show any such increase with respect to Vβ gene usage. Vβ analysis of thymocytes, carried out 3 weeks after injection of RadLV, also did not show such an increase, although gp70 expression was high (data not shown).

TCR gene rearrangement pattern in RadLV-induced lymphomas. The clonality of the tumors with respect to their TCR genes was examined through the use of probes for the Jβ1 and Jβ2 gene segment clusters (11). Both probes were hybridized

to EcoRI-digested DNA obtained from RadLV-induced lymphomas; the results are presented in Fig. 6. All of the 11 tested lymphomas showed clonal or oligoclonal Jβ2 gene rearrangements as well as variable amounts of the 2.5-kb germ line Jβ2 gene structure (Fig. 6A). Four of the lymphomas (lymphomas 1, 2, 3, and 7) also displayed clonal Jβ1 gene rearrangements, while the remainder had the germ line Jβ1 gene structure (not shown). Three lymphomas (lymphomas 7, 9, and 11), in which the expression of the TCR was exclusively Vβ6, were oligoclonal with respect to the Jβ2 TCR gene structure; one (lymphoma 7) also exhibited a clonal rearrangement of the Jβ1 gene (Fig. 6B). In the Vβ6-expressing tumors, the sizes of the rearranged Jβ2 gene differed, indicating that different recombinational events led independently to each of these tumors.

DISCUSSION

We have previously described a cell population in the thymuses of irradiated mice in which the first transformation-associated changes, and the phenotypic evolution during progression toward frank malignancy, can be detected (28, 41). It was shown that these cells express a surface glycoprotein, 1C11, also present on 10% of normal thymocytes (the immature subset) and on virtually all neoplastic T cells (28, 41). On preneoplastic and neoplastic, but not on normal immature, thymocytes, the 1C11 molecule is coexpressed with the differentiation marker CD3 of the TCR complex. The earliest identifiable preneoplastic thymocytes in these irradiated mice
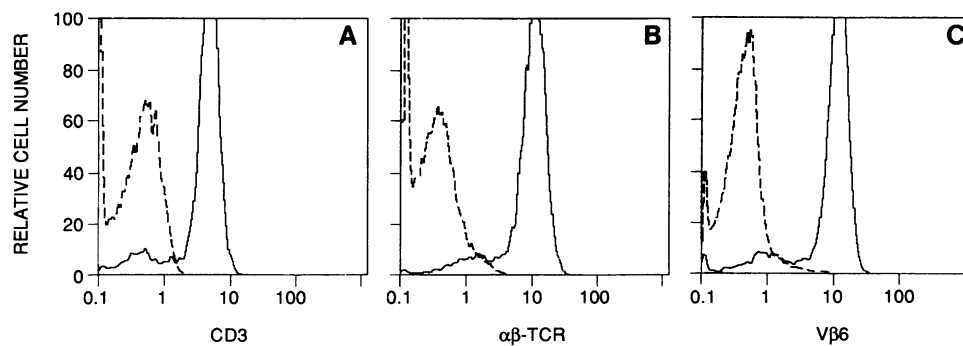

FIG. 5. Cell surface expression of CD3, αβTCR, and Vβ6 chain by a RadLV-induced primary lymphoma. The cells were stained with anti-CD3 (A), anti-αβTCR (B), or anti-Vβ6 (C) antibody followed by appropriate secondary antibodies as described in Materials and Methods. Dashed lines in each panel represent background fluorescence.

TABLE 2. Preferential expression of the Vβ6 gene product of the TCR complex by RadLV-induced thymic lymphomas[a]

RadLV-induced thymic lymphoma	% of cells expressing the indicated determinants					
	CD3	αβTCR	Vβ6	Vβ8	Vβ5	Vβ9
1	93.4	91.2	84.3	—[b]	—	—
2	67.1	76.0	—	—	—	72.2
3	67.8	68.1	—	77.8	—	—
4	50.8	46.0	40.2	—	—	—
5	78.3	ND[c]	80.7	—	—	—
6	85.6	ND	86.7	—	—	—
7	82.3	95.3	88.3	—	—	—
8	ND	ND	—	—	61.1	—
9	79.7	ND	—	—	65.8	—
10	74.3	69.5	66.8	—	—	—

[a] Twelve RadLV-induced thymic lymphomas were analyzed by FACS, using monoclonal antibodies against the following TCR Vβ elements: Vβ2, Vβ3, Vβ5, Vβ6, Vβ7, Vβ8, Vβ9, Vβ11, and Vβ13. Two lymphomas did not show reactivity against any of the anti-Vβ antibodies.

[b] —, no reactivity found.

[c] ND, not determined.

are also CD4⁻ CD8⁻; as they progress to overt lymphoma, they become CD4⁺ CD8⁺ and CD4⁻ CD8⁺ while retaining high levels of 1C11 and CD3 expression (40a).

In this report, we have characterized preneoplastic thymocytes in RadLV-infected mice to compare them and their transformation pathway with those involved in radiation lymphomagenesis. The data reveal a bimodal course of events after intrathymic injection of the virus. Viral envelope gp70 becomes detectable within a few days, and the population of virus-positive thymocytes rises rapidly. It then falls precipitously at 3 to 4 weeks, only to rise again sharply and permanently in the course of the next 2 weeks. This rebound is accompanied by a parallel increase in the population of 1C11 and CD3 cells. Soon after infection, a distinctive alteration in the pattern of CD4 and CD8 expression becomes apparent on a subset of infected thymocytes. The gp70⁺ CD3⁺ but not the gp70⁺ CD3⁻ population shows a rise in the percentage of CD4⁺ CD8⁺ cells and a decrease in that of CD4⁻ CD8⁺ cells. These results and the reported loss of cortical thymocytes at 2 to 3 weeks after infection by RadLV (6) suggest that while many thymocytes are susceptible to productive infection by the virus, most do not undergo neoplastic transformation and die intrathymically. A relatively rare thymocyte subset, infected initially or secondarily, is presumably the target for transformation. It may be the population identified by early coexpression of gp70 and CD3, which begins to expand at 4 to 5 weeks after infection. As in irradiated mice, leukemic progression is marked by increasing numbers of 1C11⁺ and CD3⁺ thymocytes. However, unlike the predominantly CD4⁻ CD8⁺ and CD4⁺ CD8⁺ radiation-induced lymphomas (40a), RadLV-induced tumors are heterogeneous with respect to their CD4 CD8 phenotype, and a significant number are CD4⁺ CD8⁻, a phenotype not observed in the former but reported in AKR virus-induced lymphomas (15) and the RadLV-binding C6VL lymphoma cell line (38).

It has been postulated that a critical step by which retroviruses cause thymic lymphomas in mice is via their binding to the cognate TCR on thymocytes, with resultant persistent stimulation and eventual transformation of the cells (32, 38). The increasing numbers of CD3⁺ thymocytes during RadLV-induced lymphomagenesis is consistent with the clonal expansion of one or more TCR Vβ-bearing populations following initiation of the transformation process. In fact, most of the tested T-cell lymphomas show clonal expansion of a particular TCR Vβ-bearing T-cell population. The presence of clonal rearrangements of both the Jβ1 and Jβ2 genes in some tumors
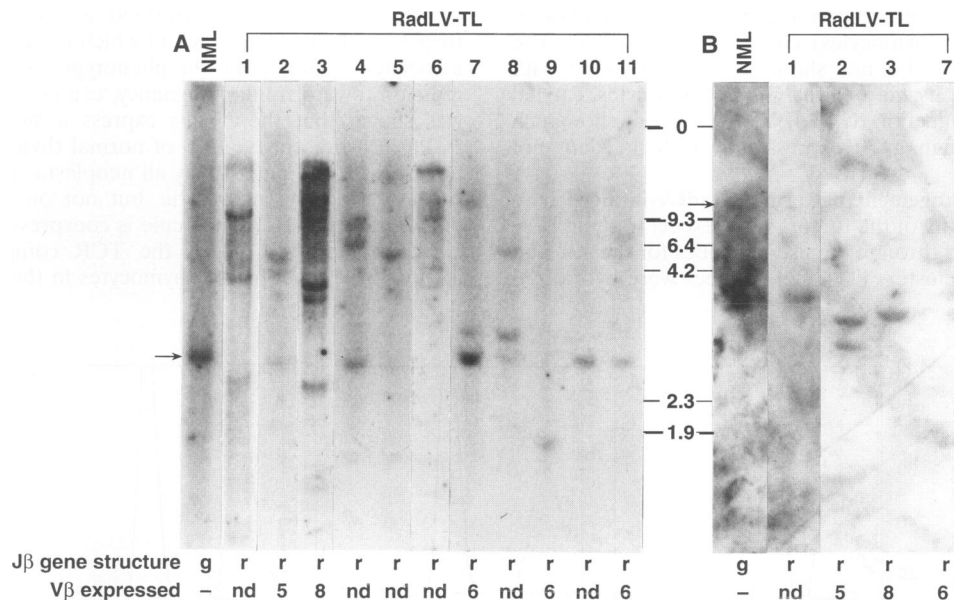

FIG. 6. Southern blot analysis of TCR β-chain gene rearrangement in RadLV-induced lymphomas. Eleven lymphoma sample DNAs and normal mouse liver (NML) DNA were isolated as described in Materials and Methods. Each sample of DNA was digested with EcoRI and hybridized with the Jβ2 (A) or Jβ1 (B) probe. The presence of germ line bands (g) and of rearranged bands (r) is indicated at the bottom. The particular Vβ chain expressed by each lymphoma, as determined by FACS analysis (see Materials and Methods), is shown as well. Except for lanes 1, 2, 3, and 7, no Jβ1-hybridized band was detected in the remaining lymphoma samples. Arrows indicate the positions of germ line bands obtained with Jβ1 and Jβ2 probes. HindIII-digested λ DNA was used as a molecular standard. Numbering of the lymphomas does not correspond to the numbers in Tables 1 and 2. Sizes are indicated in kilobases.

(Fig. 6, tumors 2, 3, 4, and 8) may reflect the nonproductive rearrangement of the Jβ1 gene, followed by a productive rearrangement of the Jβ2 gene, and does not necessarily imply the presence of more than one clone in these tumors. However, in two cases (Fig. 6, tumors 9 and 12), the appearance of multiple bands of hybridization when EcoRI-digested genomic DNA was probed with the Jβ2 gene indicates the presence of multiple, independently derived clones that in some cases (tumor 12) express the same Vβ gene (Vβ6) segment. These data support the notion that particular Vβ-expressing cells are selected in RadLV lymphomagenesis, reminiscent of the binding of endogenous and exogenous superantigens to T cells that express the same clonotypic TCR Vβ gene segment (2, 31). Thus, the oligoclonality of Vβ gene rearrangements in RadLV-induced lymphomas, in conjunction with the restricted repertoire of Vβ gene expression, could be interpreted to indicate that RadLV may contain or induce the expression of a superantigen during lymphomagenesis. Probable mechanisms could include binding of a RadLV-related antigen to the antigen recognition domain of the TCR in a subset of the Vβ6 or Vβ5 element or binding to constant domains present in all Vβ5 or Vβ6 TCR, as occurs with superantigens (2, 31). Future experiments involving the PCR amplification and sequencing of TCR sequences present in the antigen binding and β-chain constant-region domains could distinguish between these possibilities. In contrast to the preferential expression of Vβ6 TCR in RadLV-induced lymphomas and the RadLV-binding C6VL line, the Vβ8 gene product is found in the majority of radiation-induced lymphomas of C57BL/Ka mice (40a). Hence, despite many similarities, it appears that the targets of lymphoma induction by radiation and MuLV are not identical.

Preferential use of TCR V genes has been documented in some autoimmune diseases, e.g., experimental autoimmune encephalomyelitis of mice (1, 45) and experimental myasthenia gravis (19). In addition, tumor-infiltrating T cells in uveal melanoma in human (36) and Friend virus-induced tumors in mice (44) were shown to express specific TCR Vα and Vβ genes. In a recent study, Hugin et al. demonstrated that a replication-defective MuLV that induces murine AIDS encodes a superantigen that stimulates Vβ5-positive CD4$^+$ CD8$^-$ T cells (18). Our observation of the clonal expansion of a subset of Vβ-bearing T-lymphoma cells in RadLV-induced lymphomagenesis suggests that a particular set of the Vβ repertoire such as Vβ6 of the TCR may be important in the pathophysiology of the disease. In many of the autoimmune diseases mentioned above, the specificity of the antigens that cause stimulation of autoreactive T cells has been well characterized. Such precise determination of the antigenic components of RadLV that may bring about stimulation and clonal expansion of a particular Vβ-bearing T cells has not been made. Nevertheless, in light of the theory of receptor-mediated leukemogenesis, such a mechanism may well be operative through interaction between the viral envelope antigen and the Vβ subunit of the TCR. Determination of antigenic specificities of retrovirus-induced T-cell lymphomas may eventually help us to understand the biochemical basis of cellular proliferation and transformation. It would also be of interest to examine whether ablation of Vβ6$^+$ thymocytes by antibody administration would reduce the incidence or delay the appearance of virus-induced lymphomas. In this regard, it is important to note that treatment of mice with anti-Vβ8 antibody could abrogate the ability to transfer experimental autoimmune encephalomyelitis in PL/J mice (49). Clearly, the ability of various anti-Vβ TCR antibodies to alter patterns of development of MuLV-induced lymphomas should be investigated in the future.

Finally, these data describe the phenotypic changes occurring in MuLV-infected thymocytes which culminate in neoplastic transformation of T cells. Conceivably, the gp70$^+$ 1C11^{hi} CD3^{hi} subset, expressing the Vβ6 or Vβ5 element of the TCR, could constitute the probable target(s) for thymic neoplasia. If these cells, expressing the appropriate Vβ TCR, are at a stage where positive selection can occur, RadLV antigens may substitute for the positively selecting elements.

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