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AKR murine thymic leukemias are from a distinct thymic cell lineage and do not express the β chain of the T-cell antigen receptor

(DNA rearrangement/α-, β-, and γ-chain genes/*Tsu* linkage group)

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ABSTRACT **Characterization of tumors that arise spontaneously in the AKR mouse indicates that they are derived from cells of a distinct T-cell lineage. Cells in this subclass bear surface antigens, designated Tpre, Tthy, Tind, and Tsu, which are encoded by genes in the *Tsu* linkage group on murine chromosome 12. We have examined the rearrangement and expression of genes encoding the T-cell α, β, and γ chains in these tumors. Although these cells contain α-chain mRNA, they do not produce a normal-sized β-chain mRNA. Most of them also lack γ-chain mRNA. Each thymic leukemia was derived from a cell arrested at a different stage of development as defined by their expression of terminal deoxynucleotidyl transferase and Thy-1 mRNA. The data presented here are consistent with a model in which thymocytes expressing Tpre, Tthy, Tind, or Tsu undergo somatic development parallel to the development of other T cells. However, these thymocytes do not appear to differentiate into cells bearing α–β heterodimers of the T-cell antigen receptor.**

T lymphocytes all derive from stem cells that mature in the thymus via a series of developmental stages. The pathway of helper and cytotoxic T-cell differentiation is now well defined with respect to the expression of cell surface antigens. This cell lineage has been characterized by the state of rearrangement and expression of the genes encoding antigen receptors (1–6). The developmental pathways that lead to the production of other types of T cells are less well defined (5–8).

Less than 20% of murine thymocytes express the Tpre, Tthy, Tind, and Tsu surface antigens (9). These surface antigens are encoded by the *Tsu* gene linkage group adjacent to the immunoglobulin heavy chain genes on murine chromosome 12 (9). Although these antigens have been identified on the surface of functional suppressor T cells (10–12), the physiological role of other T cells that express them has not been determined. As they mature, the T cells in this subclass express these antigens in a developmentally controlled sequence. For example, Tpre is expressed on immature T cells while Tsu is found preferentially on the surface of adult T cells located in lymph nodes (13).

The tumors that arise spontaneously in AKR mice (14, 15) are derived from the subset of T cells that expresses *Tsu* linkage group antigens (9, 12). These tumors appear to arise by the malignant transformation of individual cells at various stages in the maturation pathway of T cells (16). We report here the characteristics of these thymic leukemia cells with respect to their expression of terminal deoxynucleotidyltransferase (TdT) and Thy-1. To further characterize these tumors we also investigated the state of rearrangement and expression of their T-cell antigen receptor genes (for the α and β chains) and T-cell γ-chain genes. We find that these thymic leukemia cells do not express normal-sized β-chain mRNA and, therefore, cannot express an antigen-specific receptor analogous to the one found on cytotoxic and helper T cells. We propose that these malignant T cells represent different intermediates in the developmental pathway of a T-cell subset. These cells mature over a pathway that is similar to, but distinct from, that used by cytotoxic and helper T cells.

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MATERIALS AND METHODS

Source of Thymic Leukemias. Spontaneous thymic leukemias arose in individual AKRL mice housed in the facilities of H.H. Tumors were adapted to *in vitro* growth and passaged in tissue culture media for several months. Tumor cells were subcloned and obtained as progeny of single cells (17). The tumors used in this study were chosen to represent several surface phenotypes of cells bearing *Tsu* linkage group encoded cell surface antigens. The names (and phenotypes) of these lines are AKRL 29 (expresses none of these antigens), AKRL 52 (Tthy+, Tsu+), AKRL 65T (Tthy+, Tind+, Tsu+, Tpre+), AKRL 71 (Tthy+, Tsu+, Tind+), ARKL 75 (Tthy+), AKRL 77 (Tthy+, Tind+), AKRL 79 (Tthy+, Tpre+, Tind+), AKRL 89 (Tthy+, Tsu+, Tind+), and AKRL 95 (Tthy+).

Source of DNA Probes. Mouse β-chain genes and mRNA were characterized with a human β-chain constant region probe that cross-hybridizes with the murine β-chain constant region (18). Probe for the mouse γ chain (3) was obtained by screening a cDNA library made from mRNA from the cell line MD26 (ref. 19 and C.M., unpublished data). The 700-base-pair insert includes most of the constant region (20). The cDNA encoding murine TdT was the kind gift of N. Landau and D. Baltimore (21). The mouse α-chain cDNA clone was the kind gift of M. Davis (2) as was the Thy-1 cDNA clone.

RNA Gel and Southern Blot Analyses. RNA for gel blot analyses was prepared as described (22). Twenty micrograms of RNA was electrophoresed in 1.5% denaturing agarose gels. DNA for Southern blot analyses was isolated from tissue as described (23). Ten micrograms of DNA was digested with the restriction enzymes *Bam*HI, *Eco*RI, and *Eco*RV. Digested DNA was electrophoresed in 0.9% agarose gels. λ phage DNA, digested with *Hin*dIII, was used as a molecular size marker. RNA for gel blot analyses and DNA for Southern blot analyses were transferred onto nitrocellulose and hybridized to nick-translated ^{32}P-labeled cDNA probes (24).

Abbreviation: TdT, terminal deoxynucleotidyltransferase.

RESULTS

AKR Mouse Thymic Leukemias Are Arrested at Different Stages of T-Cell Differentiation. The pathway by which cytotoxic and helper T cells mature has been characterized by determining which genes are expressed at particular stages in their development (25). AKR thymic leukemias appear to be derived from an unusual T-cell subset because they express the cell surface antigens of the *Tsu* linkage group. Each AKR thymic leukemia represents an intermediate cell arrested at a particular stage of differentiation (Fig. 1). We have characterized the pathway by which this subset of T cells matures by examining each tumor for content of particular mRNAs, and expression of particular cell surface antigens. To pinpoint the origin of the AKR thymic leukemias, we have characterized them with respect to expression of Thy-1 and TdT mRNA (Table 1 and Fig. 2). Although AKR thymic leukemias express cell surface proteins characteristic of maturing T-cells (Thy-1, Lyt1, and Lyt2), they also express the antigens Tpre, Tthy, Tind, or Tsu (9). Of the 10 thymic leukemias analyzed, we were able to stage 9 on the pathway of maturing T cells. AKRL 71 did not express the *Tsu* linkage group encoded cell surface antigens (data not shown), Thy-1, or TdT; its lineage remains uncertain.

Most AKR Tumors Express T-Cell α-Chain but Not β- or γ-Chain mRNA. Three immunoglobulin-like genes (designated α, β, and γ) rearrange during somatic differentiation of T cells (1–7). The α and β chains comprise subunits of the T-cell antigen receptor, while the role of the γ chain is uncertain. The α, β, and γ mRNA content of the AKR thymic leukemias was determined by RNA gel blot analysis (Fig. 2). All of the tumors, except AKRL 77, contain a normal-sized α-chain mRNA. However, none of the tumors contains a normal-length β-chain mRNA and most of them lack γ-chain mRNA.

Table 1. Phenotypes of AKRL leukemias

	AKRL leukemias									
	67	89	52	95	29	79	71	75	65	77
β rearrangements	+	+	+	+	+	+	+	+	+	+
β-chain mRNA	−	−	−	Ab	−	−	−	−	Ab	−
α-chain mRNA	+	+	+	+	+	+	+	+	+	−
γ rearrangements	+	+	+	+	+	+	+	+	+	−
γ-chain mRNA	−	+	+	−	−	−	−	−	−	−
Thy-1 mRNA	−	+	+	+	+	+	−	+	+	+
TdT mRNA	+	+	−	+	+	−	−	−	−	−

The mRNA content of the AKR thymic leukemias was determined by RNA gel blot analyses (Fig. 2), and the rearrangement of their β- and γ-chain genes was characterized by Southern blot analyses (Fig. 3). + and −, Presence or absence, respectively, of mRNA or DNA rearrangement; Ab, aberrant-sized mRNA.

Rearrangement of α, β, and γ Chain Genes in AKRL Tumors. Since the majority of these T cells do not contain β- or γ-chain mRNA, we asked whether their β- or γ-chain genes were rearranged. Southern blot analysis of DNA from

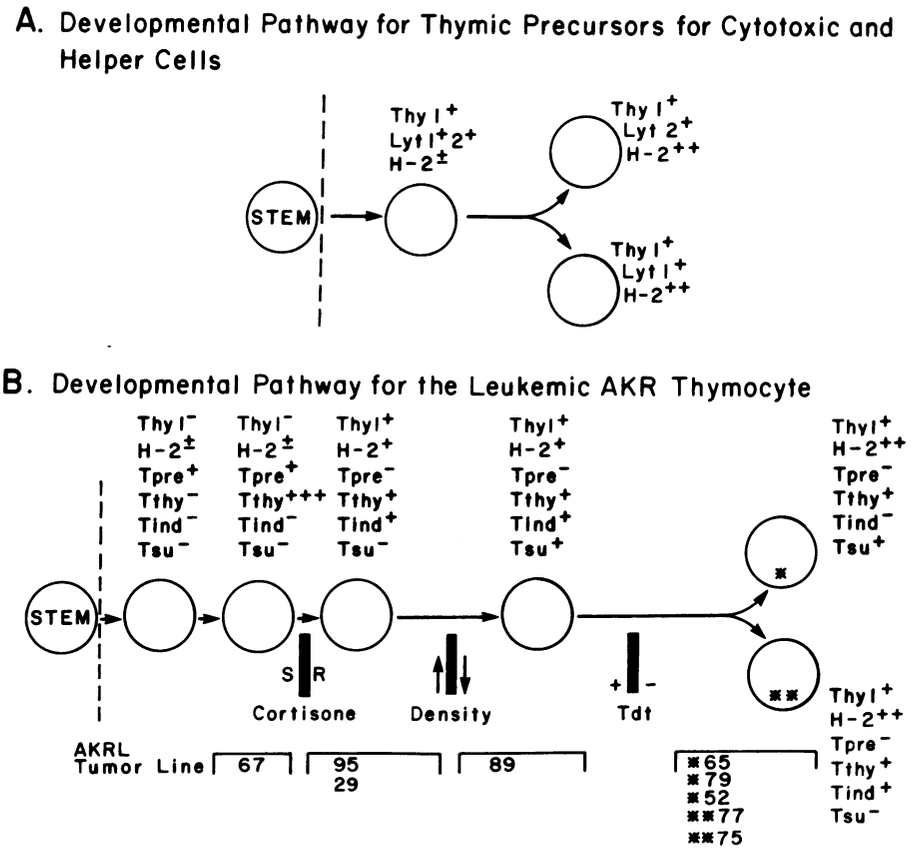

FIG. 1. The developmental pathways of cytotoxic and helper T cells (25) (*A*) and T cells expressing the *Tsu* linkage group encoded surface antigens (*B*) (9). T cells derived from pathway *A* include cytotoxic and helper T cells that do not express the *Tsu* linkage group encoded antigens Tind, Tsu, Tthy, and Tpre. Phenotypes used to determine the stage of differentiation of nonmalignant T cells expressing the *Tsu* linkage group antigens include: cortisone sensitivity (S) or resistance (R), buoyant density in bovine serum albumin gradients, and expression of H-2 antigens, TdT, and Thy-1 (9). The developmental stage of individual AKR tumors was assigned on the basis of their surface expression of the *Tsu* linkage group encoded antigens (6). The presence or absence of the various cell surface antigens is indicated by + or −. The expression of TdT and Thy-1 mRNAs found in each tumor (Table 1 and Fig. 2) is consistent with its developmental stage. Mature T cells expressing the *Tsu* linkage group antigens have either Tthy and Tsu or Tthy and Tind on their surfaces. These two phenotypes are designated * or **. AKRL 71 is not included in this pathway because it does not express any of the *Tsu* linkage group antigens on its surface.

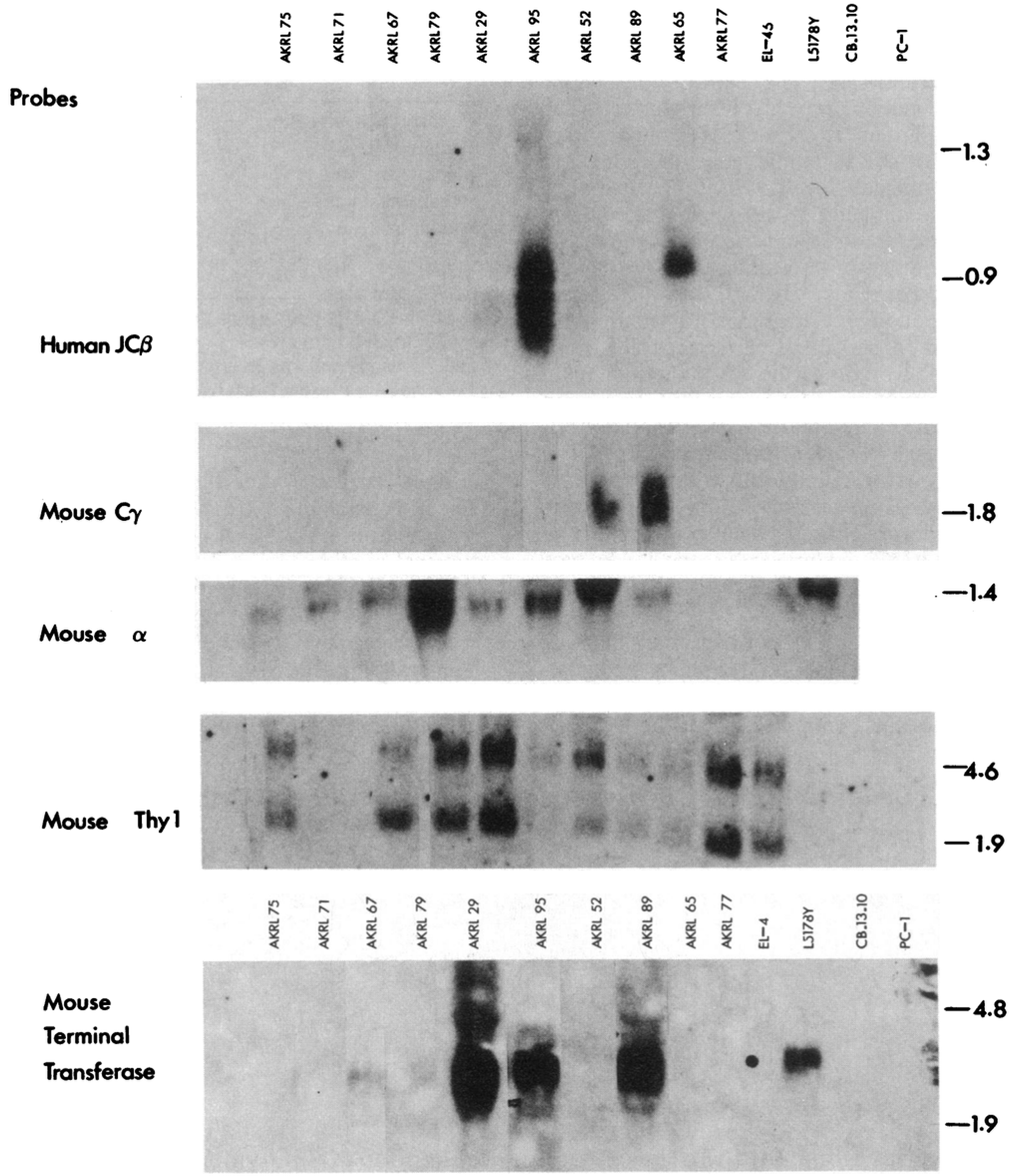

FIG. 2. RNA gel blots of AKRL tumor mRNAs hybridized to α-, β-, and γ-chain, TdT, and Thy-1 ^{32}P-labeled cDNA probes. Lanes containing RNA derived from B-cell lines CB.13.10 and PC-1 and from T-cell lines EL-4, EL-45 (a variant of EL-4), and L5178Y were also hybridized to the cDNA probes. The size of each mRNA species (in kilobases) was determined by comigration with 18S and 28S rRNA. Normal β-chain mRNA (1.3 kilobases) was not found in any of the tumors; abnormal mRNA (0.9 kilobases) is present in tumors AKRL 65 and AKRL 95. The mRNA content of each cell is summarized in Table 1.

each of the 10 AKR thymic leukemias with DNA probes derived from the constant region segment of β-chain and γ-chain genes showed that all tumors rearranged their β-chain genes while 9 of 10 tumors contained rearranged γ-chain genes (Fig. 3). One tumor, AKRL 95, did not appear to contain rearranged γ-chain genes after digestion of the DNA with either *Eco*RI or *Eco*RV (Fig. 3 and data not shown). Further, most tumors have rearranged their γ- and β-chain genes on both chromosomes, because restriction fragments corresponding to germ-line DNA are missing (Fig. 3). Because the β- and γ-chain genes have rearranged in most of the tumors, we might have expected these genes to be transcribed. However, most of these cells contain neither β-chain nor γ-chain mRNA.

DISCUSSION

In an earlier communication (12), we reported that AKRL tumors express *Tsu* linkage group encoded cell surface antigens and postulated that these tumors represent a subset of thymocytes that has undergone transformation. Here we showed that these tumors have undergone rearrangements of their α-, β-, and γ-chain genes. These tumors cannot express the α–β heterodimer of the T-cell antigen receptor, since they do not contain normal-length β-chain mRNA. Although each T cell contains at least one rearranged β-chain gene, these rearranged genes fail to produce mRNA. One possible explanation for the lack of β-chain mRNA is that these tumors lost their ability to produce this mRNA after malignant transformation. However, many other tumors, including radiation-induced and retroviral-associated tumor lines, express β-chain mRNA after many years in culture (5). Therefore, we believe that the absence of β-chain mRNA in these AKR tumor-derived cells is not due to the process of malignant transformation. Most probably, the AKR thymic leukemia cells derive from malignant transformation of T cells that belong to a type of T cell that never contains functional β-chain mRNA. The only other T cells known to

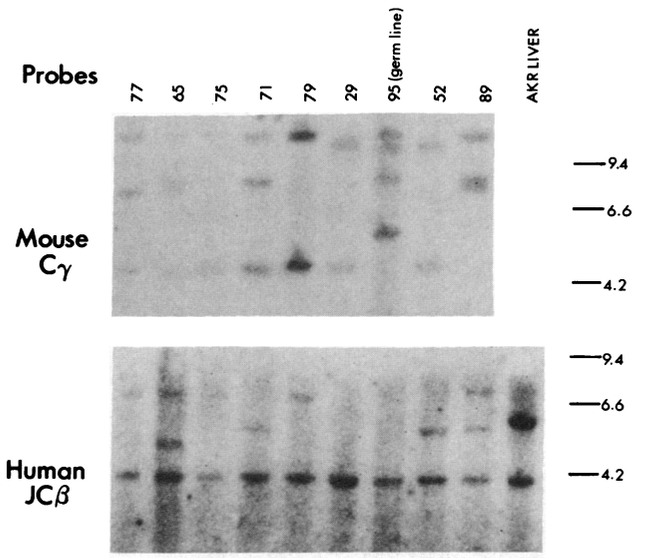

FIG. 3. Southern blot analyses of AKRL tumor DNAs hybridized to γ (*Upper*) and β (*Lower*) ^{32}P-labeled DNA probes. DNAs were digested with restriction enzymes *Eco*RV (*Upper*) and *Bam*HI (*Lower*). The mobilities of bacteriophage λ-derived *Hin*dIII fragments (in kilobase pairs) are indicated. AKRL tumor 95 DNA (germ-line) produced a pattern that was identical to AKR liver DNA when hybridized to the γ-chain probe (data not shown). The state of rearrangement of the β- and γ-chain genes in each tumor DNA is summarized in Table 1.

lack β-chain mRNA are T-cell hybridomas with suppressor function (5, 6). All cytotoxic and helper T-cell clones express β-chain mRNA (1, 26), as do their thymic precursor cells (27). Therefore, we propose that the AKR thymic leukmias are derived from a T-cell lineage distinct from the lineage of T cells that produces cytotoxic and helper cells.

The functional role of T cells represented by the AKR tumors remains uncertain. Nine of the 10 characterized AKR thymic leukemia cells express *Tsu* linkage group cell surface antigens. These cell surface antigens have been found on the surface of suppressor T cells, but not on the surfaces of cytotoxic or helper T cells. We expect that cells in this T-cell lineage express a cell surface antigen specific receptor that is different from the α- and β-chain bearing antigen receptor.

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