Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 3700–3704, May 1991
Immunology

Thymic targets for Abelson murine leukemia virus are early γ/δ T lymphocytes

(T cells/T-cell receptor genes/v-*abl* oncogene)

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ABSTRACT **Molecular analysis has shown that the majority of Abelson murine leukemia virus (Ab-MuLV)-induced primary thymomas represent transformed γ/δ thymocytes. Many of these thymomas are of monoclonal origin as judged by provirus integration pattern and contain rearranged genes encoding T-cell receptor (TCR) γ and δ chains but germ-line immunoglobulin heavy-chain genes. Some of the monoclonal tumors contain multiple rearranged alleles encoding TCR γ, δ, and β chains. Further, one Ab-MuLV thymoma cell line contained germ-line-configuration TCR γ- and δ-chain genes, which became rearranged after *in vitro* propagation. Clones of this cell line were observed to rearrange these genes after intrathymic passage. Also, some subclones of this cell line underwent rearrangement of their immunoglobulin heavy-chain genes in culture. These observations suggest that the thymic targets for Ab-MuLV transformation are early γ/δ thymocytes, some of which continue to rearrange their TCR γ- and δ-chain genes.**

The B and T lymphocytes of the immune system can be distinguished by their antigen receptors, which respectively are immunoglobulin (Ig) receptors and the α/β (1) or γ/δ (2) T-cell antigen receptors (TCRs). These moieties are encoded in the germ line by the Ig heavy (H)-chain (Ig_H) and Ig light (L)-chain (Ig_L) genes and by the TCR α-, β-, δ-, and γ-chain genes [TCR α, β, γ, and δ genes (*Tcra, Tcrb, Tcrg*, and *Tcrd* in mouse gene nomenclature)]. These genes are similar in organization and in the necessity for gene rearrangement to juxtapose variable (V) region, diversity (D) region, and joining (J) region genes to generate protein V regions. During B-cell differentiation, Ig gene rearrangement is thought to occur in a temporal order such that D_H-to-J_H joining occurs before V_H-to-D_H joining (3, 4). Ig_L genes are then rearranged after V_H-to-D_H–J_H joining (5). Rearrangement of TCR genes is believed to occur in an analogous manner (6).

Much of the current understanding of the mechanisms of Ig gene rearrangement has come from study of Abelson murine leukemia virus (Ab-MuLV)-transformed B-cell lines. Ab-MuLV transformants typically represent the earliest stages of B-cell development in which D_H-to-J_H or V_H-to-D_H–J_H joining has occurred (4).

In addition to inducing B-cell tumors, Ab-MuLV rapidly and efficiently induces thymomas after intrathymic injection of the virus (7–9). Molecular analysis of Ab-MuLV thymoma cell lines has shown that some were clearly T-cell lines, being Thy-1^+ and containing rearranged TCR β and γ genes but germ-line Ig_H genes (9, 10). Others were B-cell lines, being Thy-1^- and containing rearranged Ig_H and Ig_L genes but germ-line TCR β and γ genes (10). However, some Ab-MuLV thymoma cell lines were of mixed phenotype, expressing low levels of Thy-1 and containing rearranged Ig_H, TCR γ, and TCR β genes (10). Thus, it appears that several thymic cell types can be targets for Ab-MuLV thymomagenesis.

To clarify the nature of the thymic targets for Ab-MuLV, we have examined Ab-MuLV primary thymomas and thymoma cell lines for the configurations of their TCR and Ig_H genes. We have found that Ab-MuLV thymomas and their cell lines represent transformed early γ/δ thymocytes. One thymoma cell line was found to rearrange its germ-line configuration TCR γ and δ genes.

MATERIALS AND METHODS

Animals. C57BL/6ByJ (B6) mice were obtained from our colonies or were purchased from The Jackson Laboratory.

Virus Preparation. Helper virus-free Ab-MuLV pseudotyped in ψ-2 cells [Ab-MuLV(ψ-2)] and helper virus-containing Ab-MuLV pseudotyped with helper Moloney murine leukemia virus [Mo-MuLV; Ab-MuLV(Mo-MuLV)] stocks were prepared and titered as described (11).

Tumor Induction. For primary thymoma induction, weanling B6 mice were injected intrathymically with 2×10^4 to 1×10^5 focus-forming units of Ab-MuLV(ψ-2) or Ab-MuLV(Mo-MuLV). Thymoma cell lines were passaged *in vivo* by intrathymic injection of 1×10^6 cells into weanling B6 mice.

Tumor Cell Culture. Thymomas were excised and were disaggregated by perfusion. The cells were resuspended and maintained at a density of 2.5×10^5 cells per ml in RPMI 1640 medium supplemented with 20% (vol/vol) fetal bovine serum, 50 μM 2-mercaptoethanol, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Cells were cloned at limiting dilution by plating at 0.3 cell per well in 96-well microtiter plates.

Fluorescence-Activated Cell Sorter (FACS) Analysis. FACS analysis was performed as described (12).

DNA Preparation and Southern Blotting. Chromosomal DNA (10 μg) was digested to completion with restriction endonucleases. The DNA fragments were resolved by electrophoresis through 0.7% agarose gels, transferred to nitrocellulose filters, and hybridized to random primer-labeled probes.

Nucleic Acid Probes. Probes derived from the TCR $J_{\gamma 1}$, $V_{\gamma 2}$, $V_{\gamma 4}$, and $J_{\delta 1}$ genes were prepared as described (13, 14). The

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Abbreviations: Ab-MuLV and Mo-MuLV, Abelson and Moloney murine leukemia viruses; TCR, T-cell receptor; Ab-MuLV(Mo-MuLV), Ab-MuLV pseudotyped with helper Mo-MuLV; Ab-MuLV(ψ-2), helper virus-free Ab-MuLV pseudotyped in ψ-2 cells; B6, C57BL/6ByJ; H, heavy; L, light; V, variable; D, diversity; J, joining; C, constant; TCR α, β, γ, and δ genes, TCRα-, β-, γ-, and δ-chain genes; Ig_H and Ig_L, immunoglobulin heavy and light chains; FACS, fluorescence-activated cell sorter.

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$J_{\gamma 1}$ probe cross-hybridizes with the $J_{\gamma 2}$ and $J_{\gamma 3}$ genes (13, 14). Assignment of $J_{\gamma 1}$ or $J_{\delta 1}$ probe-hybridizing DNA fragments to germ-line or rearranged configurations was made as described (13, 15–17). The TCR γ-chain constant (C) region probe $C_{\gamma 4}$ was the 0.43-kilobase (kb) *Ava* II fragment of the clone 5/10–13 1.2 (18). The TCR $C_{\beta 1}$ probe was the 0.8-kb *Pst* I fragment of pRBL-5 (19). The Ig J_H probe was the 3.8-kb *Xba* I fragment of pRI-JH (5), and the V_H7183 gene family probe was the 0.28-kb *Pst* I–*Eco*RI fragment of pV_H81x (20). The *abl*-specific probe was the 1.9-kb *Hin*dIII–*Sac* I fragment of pSA17 (21).

RESULTS

Helper Virus-Free Ab-MuLV Rapidly and Efficiently Induces Thymomas. We and others (22) have observed that Ab-MuLV(ψ-2) efficiently induces thymomas in weanling B6 mice; 31 of 64 (48%) mice injected intrathymically with Ab-MuLV(ψ-2) developed thymomas 22–50 days after injection. The Ab-MuLV proviral integration patterns of 17 of these tumors and of eight thymomas induced by Ab-MuLV(Mo-MuLV) were analyzed by digestion of the DNAs with *Eco*RI, which permits the detection of unique proviral integration fragments (11). Hybridization of these DNAs with an *abl*-specific probe showed that each thymoma contained one to four unique Ab-MuLV proviral integration sites (data not shown).

Configuration of the TCR and Ig_H Genes in Ab-MuLV Primary Thymomas and Thymoma Cell Lines. To determine the cellular origin of Ab-MuLV thymomas, we examined the configurations of the TCR γ, δ, and β genes and the Ig_H genes of the 25 Ab-MuLV primary thymomas and of two thymoma cell lines by Southern blot analysis. This analysis showed that the DNAs of 24 of 25 primary thymomas and of one of two thymoma cell lines contained rearranged TCR γ genes (Table 1). These TCR γ gene rearrangements were primarily of the V_6–J_1, V_2–J_2, and V_4–J_1 types (Fig. 1*A*). Also, the DNAs of 5 of 25 thymomas showed a prominent rearranged fragment of 5.2 kb (Fig. 1*A*, lanes 1, 3, and 5). The DNAs of 11 other thymomas and one thymoma cell line also showed submolar rearranged fragments of 5.2-kb (Fig. 1*A*, lane 6). Because these 5.2-kb fragments are comparable in size to the 5.0-kb V_7–J_1 TCR γ gene rearrangement seen in the BW 5147 thymoma cell line (15), they may also represent V_7–J_1 rearrangements. Submolar fragments representing TCR γ genes in germ-line configuration were also observed in the thymoma DNAs and may have been derived from contaminating normal tissue (Fig. 1*A*, lanes 1 and 5–7). Hybridization of *Eco*RI-digested DNAs of 6 thymomas and one thymoma cell line with a $C_{\gamma 4}$ probe showed that the $C_{\gamma 4}$ genes of these DNAs were rearranged (data not shown). It is not known which V_γ gene was used in these rearrangements. $V_{\gamma 7}$–$J_{\gamma 1}$, $V_{\gamma 6}$–$J_{\gamma 1}$, $V_{\gamma 4}$–$J_{\gamma 1}$, $V_{\gamma 2}$–$J_{\gamma 2}$, and $C_{\gamma 4}$ rearrangements are all observed in γ/δ thymocytes of adult mice (14–16, 23). In summary, 24 of 25 Ab-MuLV primary thymomas and one of two thymoma cell lines showed multiple rearrangements of their functional TCR γ genes.

Table 1. Summary of TCR γ, δ, and β gene and Ig_H gene rearrangements in Ab-MuLV primary thymomas and thymoma cell lines

	Rearrangements of genes					
	TCR					
Thymoma (*n*)	γ	δ	β	γ/δ	$\gamma/\delta/\beta$	Ig_H
Primary						
Ab-MuLV(ψ-2) (17)	16/17	16/17	14/17	16/17	14/17	3/17
Ab-MuLV(Mo-MuLV) (8)	8/8	7/8	7/8	7/8	6/8	2/8
Cell line						
Ab-MuLV(ψ-2) (2)	1/2	1/2	0/2	1/2	0/2	1/2

Rearrangements of TCR γ, δ, and β and Ig_H genes were detected by nucleic acid hybridization with $J_{\gamma 1}$, $J_{\delta 1}$, $C_{\beta 1}$, and Ig_H *J*-region probes, respectively.

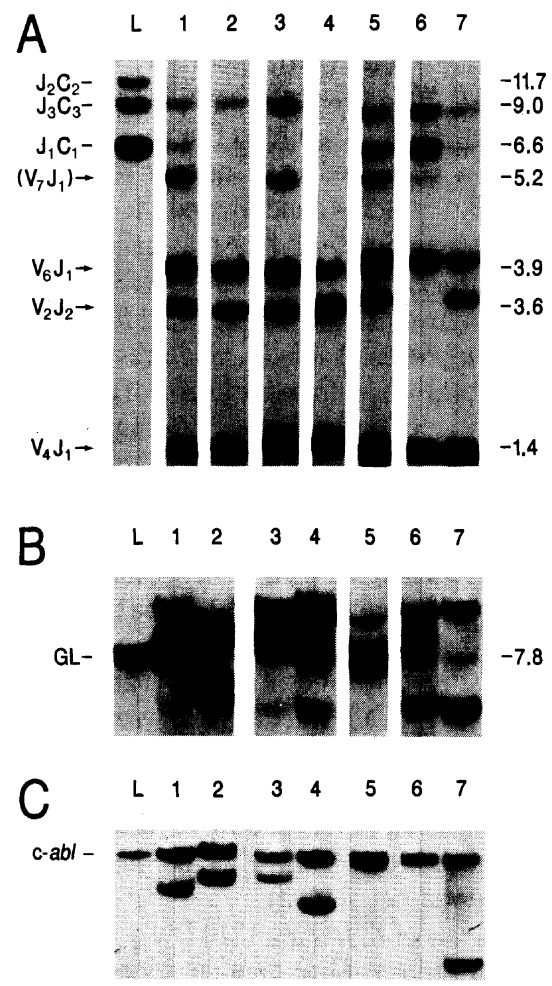

FIG. 1. Southern blot analysis of TCR γ and δ gene configurations and of proviral integration patterns in monoclonal Ab-MuLV primary thymomas. (*A*) Analysis of *Hin*dIII-digested DNAs with a $J_{\gamma 1}$ probe. Germ-line TCR γ gene fragments are indicated by lines; rearranged TCR γ gene fragments are indicated by arrows. Parentheses indicate putative V_7–J_1 TCR γ gene rearrangements. Nomenclature of TCR γ genes follows that of Heilig and Tonegawa (16). (*B*) Analysis of *Eco*RI-digested DNAs with a $J_{\delta 1}$ probe. The germ-line (GL) TCR δ gene fragment is indicated. The sizes in kb of the DNA fragments are indicated to the right of *A* and *B*. (*C*) Analysis of *Eco*RI-digested DNAs with an *abl*-specific probe. The position of the c-*abl* gene fragment is indicated. Lanes: L, normal B6 mouse liver; 1–7, independent Ab-MuLV primary thymomas.

Further Southern blot analysis showed that the DNAs of 23 of 25 thymomas and one of two thymoma cell lines contained *V*–*D*–*J*-rearranged TCR δ alleles (Table 1). Characteristic results are shown in Fig. 1*B*, lanes 1–7. The sizes of all observed rearranged TCR δ gene fragments are consistent with their representing *V*–*D*–*J* TCR δ gene rearrangements rather than the 6.6-kb D_1–D_2–J_1 or 6.9-kb D_2–J_1 TCR δ gene rearrangements observed in adult thymocytes (17). Hybridization of the *Hin*dIII-digested thymoma and thymoma cell line DNAs with a TCR $C_{\beta 1}$ probe showed that 21 of 25 thymomas contained rearranged TCR β_1 genes (Table 1). In addition, hybridization of the *Eco*RI-digested thymoma and thymoma cell line DNAs with a J_H probe showed that the Ig_H genes of 20 of 25 thymomas and one of two thymoma cell lines were in germ-line configuration (Table 1). We interpret these results to indicate that the majority of Ab-MuLV primary thymomas represent transformed γ/δ thymocytes.

A single site of Ab-MuLV provirus integration was observed in 12 thymomas (Fig. 1*C*, lanes 2–7), indicating that the bulk of these tumors descended from a single infected cell. (The samples in lanes 5 and 6 of Fig. 1*C* contained a single provirus that was detected by hybridization of *Hin*dIII-digested DNAs with the *abl* probe.) If these tumors had arisen by viral transformation of a cell that had completed

rearrangement of its TCR genes, then they would be expected to have one or two clonal rearranged TCR γ, δ, and β alleles. Instead, the DNAs of all of these thymomas contained multiple rearranged TCR γ alleles (Table 2). The DNAs of 8 of 12 thymomas contained three or four rearranged TCR δ alleles (Fig. 1*B*, lanes 3, 4, and 6), and the DNAs of 4 of 12 thymomas contained three to five rearranged TCR β alleles (Table 2). Further, the DNAs of 3 of 8 thymomas that contained multiple rearranged TCR γ and δ alleles contained multiple TCR β alleles (Table 2). Such results indicate that these thymomas arose by transformation of early thymocytes whose progeny underwent further rearrangements of their TCR γ and δ genes and in some cases of their TCR β genes.

Continuing Rearrangement of TCR γ and δ Genes. We also observed that cell lines derived from one thymoma were in the process of rearranging their TCR γ genes. Southern blot analysis of the DNAs of thymoma 1210 and the derivative cell line 1210c1 showed that their TCR γ loci were in germ-line configuration (Fig. 2*A*, lanes 1 and 2; Table 1). However, examination of 1210c1 after 42 days of *in vitro* culture showed that a submolar fragment representing a $V_{\gamma4}$–$J_{\gamma1}$ rearrangement was present in its DNA (Fig. 2*A*, lane 3). Hybridization of the *Eco*RI-digested DNAs of both 1210c1 isolates with the $C_{\gamma4}$ probe showed that their $C_{\gamma4}$ loci were in germ-line configuration (data not shown).

Further analysis of thymoma 1210 DNA showed that both TCR δ loci were in germ-line configuration (Fig. 2*B*, lane 1; Table 1). However, examination of the DNAs of both 1210c1 isolates showed that these cell lines had one TCR δ allele rearranged in D_1–D_2–J_1 configuration and the other in germ-line configuration (Fig. 2*B*, lanes 2 and 3). These results further indicated that 1210c1 was in the process of rearranging its TCR γ and δ genes.

Hybridization of 1210 and both 1210c1 DNAs with the J_H probe showed that the *J* region of the sole Ig_H locus of these cells was rearranged to form a 3.0-kb *Eco*RI fragment (Table 1). FACS analysis of the 1210c1 cell lines showed that their cell surface phenotypes were $B220^+$, Thy-$1^{low/-}$, Ly-1^-. These cell lines also expressed the AA4 antigen, a marker of hemopoietic progenitor cells.

Clonal cell lines were isolated from the 1210c1 cell line (day 42 isolate) by limiting dilution. Southern blot analysis of the DNAs of 16 of these clones showed that they were of three types, exemplified by clones 1210.2, 1210.6, and 1210.16. 1210.2 had one rearranged TCR γ locus and one germ line (V_4–J_1/GL) (Fig. 2*A*, lane 4), 1210.6 had its TCR γ loci in germ-line configuration (GL/GL) (Fig. 2*A*, lane 5), and 1210.16 had both of its TCR γ loci rearranged (V_4–J_1/V_4–J_1) (Fig. 2*A*, lane 6). Hybridization of *Eco*RI-digested 1210.2, 1210.6, and 1210.16 DNAs with the $C_{\gamma4}$ probe showed submolar rearranged fragments in 1210.2 and 1210.16, while both $C_{\gamma4}$ loci of 1210.6 were in germ-line configuration (data not shown).

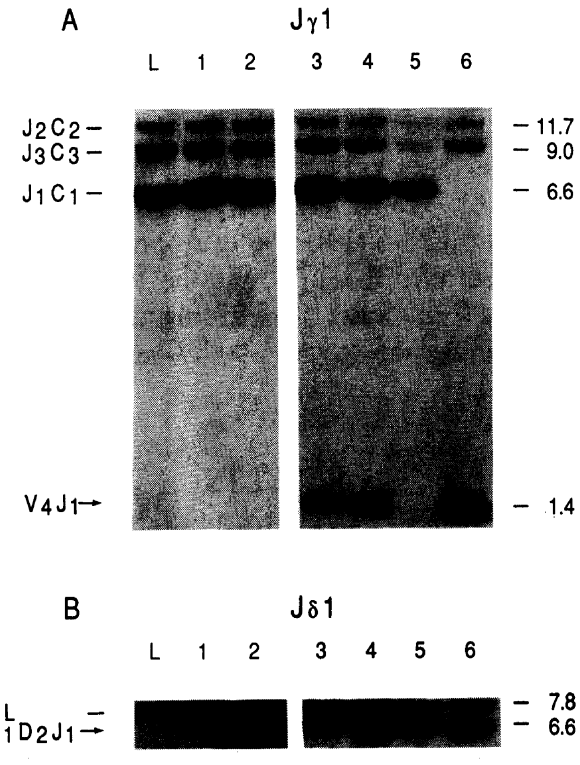

FIG. 2. Southern blot analysis of TCR γ and δ gene configuration in Ab-MuLV primary thymoma 1210 and derivative cell lines. (*A*) Analysis of *Hin*dIII-digested DNAs with a $J_{\gamma1}$ probe. Germ-line TCR γ gene fragments are indicated by lines, and rearranged TCR γ gene fragments are indicated by arrows. (*B*) Analysis of *Eco*RI-digested DNAs with a $J_{\delta1}$ probe. The germ-line (GL) TCR δ gene fragment is indicated. The sizes in kb of the DNA fragments are indicated to the right of *A* and *B*. Lanes: L, normal B6 mouse liver; 1, Ab-MuLV primary thymoma 1210; 2, Ab-MuLV thymoma cell line 1210c1; 3, Ab-MuLV thymoma cell line 1210c1 after 42 days of *in vitro* culture; 4, clone 1210.2; 5, clone 1210.6; 6, clone 1210.16.

All three clones were like the parent 1210c1 for their TCR δ gene configuration (D_1–D_2–J_1/GL) (Fig. 3*B*, lanes 4–6) and for their Ig_H gene configuration (data not shown). Hybridization of *Hin*dIII-digested 1210c1, 1210.2, 1210.6, and 1210.16 DNAs with a TCR $C_{\beta1}$ probe showed that their TCR β_1 genes were in germ-line configuration (data not shown). Further Southern blot analysis with an *abl*-specific probe of *Eco*RI-digested 1210c1, 1210.2, 1210.6, and 1210.16 DNAs showed characteristic patterns of proviral integration. 1210c1 contained *abl*-hybridizing proviral junction fragments of 12, 14, and 19.5 kb, 1210.2 and 1210.6 contained *abl*-hybridizing fragments of 12 and 14 kb, while 1210.16 contained *abl*-hybridizing fragments of 12 and 19.5 kb (data not shown).

Further Rearrangement of TCR γ and δ Genes and of Ig_H Genes After Intrathymic Growth of 1210c1 Cloned Cell Lines. To determine if 1210c1 could undergo further rearrangements of its TCR genes, we injected 1210.2, 1210.6, or 1210.16 cells into the thymuses of weanling B6 mice. To insure that the resultant secondary tumors and their cell lines were derived from the injected cells, Southern blot analysis with the *abl*-specific probe was performed on the DNAs. This analysis showed that each of the tumors and tumor cell lines had the same patterns of proviral integration as the injected cells (data not shown). Southern blot analysis of the TCR δ genes of 1210.2 (TCR δ: D_1–D_2–J_1/GL) thymomas and their cell lines showed that one of five tumors and its cell line had

Table 2. Summary of TCR γ, δ, and β gene rearrangements in monoclonal Ab-MuLV primary thymomas

Ab-MuLV primary thymomas	Rearranged TCR gene alleles,* no.		
	γ	δ	β
Ab-MuLV(ψ-2)			
1	4	2	3
2	3	3	2
3	4	4	3
4	4	3	5
5	4	2	2
6	4	3	2
7	4	4	2
Ab-MuLV/Mo-MuLV			
1	3	2	2
2	3	3	0
3	3	2	1
4	4	4	2
5	4	3	3

Tumor DNAs were digested with *Eco*RI and were examined by Southern blotting with an *abl*-specific probe for the presence of Ab-MuLV proviral integration fragments as described (11). Tumors whose DNAs showed a single Ab-MuLV provirus were deemed to be monoclonal in origin.

*The numbers of TCR γ, δ, and β alleles were detected by nucleic acid hybridization with $J_{\gamma1}$, $J_{\delta1}$, and $C_{\beta1}$ probes, respectively.

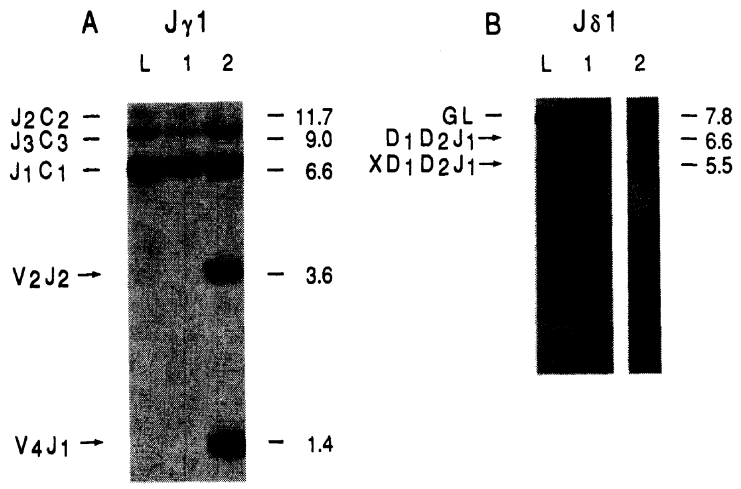

FIG. 3. Southern blot analysis of TCR γ and δ gene rearrangements in 1210c1 clones 1210.6 (*A*) and 1210.16 (*B*) after intrathymic passage. (*A*) *Hin*dIII-digested DNAs were analyzed with a $J_{\gamma 1}$ probe. Germ-line TCR γ gene fragments are indicated by lines; rearranged TCR γ gene fragments are indicated by arrows. Lanes: L, normal B6 mouse liver; 1, clone 1210.6; 2, clone 1210.6 thymoma. (*B*) *Eco*RI-digested DNAs were analyzed with a $J_{\delta 1}$ probe. The germ-line (GL) TCR δ gene fragment is indicated. Lanes: L, normal B6 mouse liver; 1, clone 1210.16 thymoma cell line; 2, subclone of clone 1210.16 thymoma cell line shown in lane 1. The sizes in kb of the DNA fragments are indicated to the right of *A* and *B*.

further rearranged the D_1–D_2–J_1 δ allele (Table 3). Because this allele could no longer be detected in the DNA by the $J_{\delta 1}$ probe, we deduced that the secondary rearrangement involved replacement of the $J_{\delta 1}$ gene with the $J_{\delta 2}$ gene or an unknown DNA sequence or involved a *V*-to-*J* rearrangement of the TCR α locus. Southern blot analysis of the TCR γ genes of 1210.6 (TCR γ: GL/GL) thymomas and thymoma cell lines showed that two of six tumors and their cell lines had rearranged their TCR γ loci (Table 3). These TCR γ gene rearrangements were of the V_4–J_1 and V_2–J_2 types (Fig. 3*A*, lane 2). Finally, analysis of 1210.16 (TCR δ: D_1–D_2–J_1/GL) tumors and cell lines showed that two of three tumors and their cell lines had further rearranged their D_1–D_2–J_1 TCR δ allele (Fig. 3*B*, lane 1; Table 3). From the size of the rearranged fragments (5.5 kb), we deduced that these secondary rearrangements most likely utilized a V_δ gene, rather than being the result of the deletion of either the 11-bp $D_{\delta 1}$ or 16-bp $D_{\delta 2}$ (17) genes from the 6.6-kb D_1–D_2–J_1 TCR δ allele. However, the 5.5-kb *X*–D_1–D_2–J_1 TCR δ gene fragment did not hybridize with any of the known V_δ genes and therefore represents the joining of either a novel V_δ gene, a V_α gene, or an unknown DNA sequence to the D_1–D_2–J_1 TCR δ allele.

We subcloned the *X*–D_1–D_2–J_1 TCR δ gene-rearranged 1210.16 thymoma cell lines by limiting dilution. We found that the TCR δ genes of five of nine subclones were in the *X*–D_1–D_2–J_1/GL configuration (Fig. 3*B*, lane 2). We also found that two of five *X*–D_1–D_2–J_1 TCR δ gene-rearranged subclones had undergone secondary rearrangements of their sole Ig_H locus (data not shown). Hybridization of these DNAs with the V_H81x probe showed that both secondary rearrangements joined a member of the V_H7183 gene family (20) to the previously rearranged J_H region (data not shown). These results indicate that 1210c1 clones can rearrange their Ig_H genes by utilizing V_H genes.

***In Vitro* Propagation of Clones 1210.2, 1210.6, and 1210.16.** To determine if the TCR and Ig_H gene rearrangements observed after intrathymic growth of 1210.2, 1210.6, and 1210.16 occurred spontaneously or because the thymus provided a favorable environment for the outgrowth of TCR and Ig_H gene-rearranged cells, we cloned the 1210.2, 1210.6, and 1210.16 cells by limiting dilution and propagated them *in vitro* for 28 days. Analysis of the subclone DNAs showed that none of the nine 1210.2 subclones, 19 1210.6 subclones, and 19 1210.16 subclones underwent further rearrangement of their TCR γ or δ genes (Table 3). A secondary rearrangement of the sole Ig_H allele was observed in 1 of 19 1210.16 subclones (Table 3) and was found to utilize a V_H7183 gene family member (data not shown). Thus, it appears that the 1210c1 clones rarely undergo spontaneous rearrangement of their TCR or Ig_H genes. Therefore, the thymic environment may supply signals that induce rearrangement of TCR genes or may permit the outgrowth of rare TCR gene-rearranged cells.

DISCUSSION

Molecular analysis of Ab-MuLV thymomas and thymoma cell lines suggests that Ab-MuLV thymomagenesis occurs by transformation of early γ/δ thymocytes. Support for this hypothesis comes from our observation that 23 of 25 primary thymomas contained rearranged TCR γ and δ alleles. In particular, 8 of 12 thymomas that were monoclonal contained multiple rearranged TCR γ and δ alleles. These patterns of TCR gene rearrangement indicate that these thymomas arose from very early thymocytes whose progeny could undergo rearrangements of their TCR γ and δ genes and in some cases their TCR β genes. Further support for this conclusion comes from the study of the 1210 thymoma and its derivative cell lines. Cloned derivatives of the 1210c1 cell line were observed to undergo rearrangement of their germ-line configuration TCR γ and δ genes after intrathymic propagation. We interpret these results to indicate that the 1210c1 cell line represents the transformed analogue of a very early thymocyte predisposed to rearranging its TCR γ and δ genes.

We also observed that subclones of the 1210c1 cell line could undergo *V*-to-*D*–*J* rearrangement of their Ig_H genes. These subclones may rearrange both TCR and Ig genes because the process of transformation has abrogated the constraints that normally confine TCR and Ig gene rearrangements to cells of the appropriate lineages. Indeed, rearranged Ig_H genes are often found in T-cell lines and tumors (24). Alternatively, 1210c1 may represent a lymphoid cell type that has both TCR and Ig genes accessible for rearrangement. It is not known if there is a normal counterpart of this cell type.

Because we observed rearranged TCR β, γ, and δ alleles in most Ab-MuLV thymomas, assignment of these cells to the

Table 3. Summary of TCR γ and δ gene and Ig_H gene rearrangements after intrathymic and *in vitro* propagation of Ab-MuLV thymoma cell line 1210c1 clones 1210.2, 1210.6, and 1210.16

			Rearrangements of TCR and Ig_H genes					
	TCR genes		After intrathymic growth			After *in vitro* subcloning		
Clone	γ	δ	γ	δ	Ig_H	γ	δ	Ig_H
1210.2	V_4–J_1/GL	D_1–D_2–J_1/GL	0/5	1/5	0/5	0/9	0/9	0/9
1210.6	GL/GL	D_1–D_2–J_1/GL	2/6	0/6	0/6	0/19	0/19	0/19
1210.16	V_4–J_1/V_4–J_1	D_1–D_2–J_1/GL	0/3	2/3	1/3*	0/19	0/19	1/19

Rearrangements of TCR γ and δ genes and Ig_H genes were detected by nucleic acid hybridization with $J_{\gamma 1}$, $J_{\delta 1}$, and Ig_H *J*-region probes, respectively.

*The Ig_H gene rearrangements observed in two subclones of the cell line derived from this tumor were not detected in either the tumor or the derivative cell line.

γ/δ thymocyte lineage is not unequivocal. Our finding that all Ab-MuLV thymomas that contained rearranged TCR β alleles also contained rearranged TCR γ and δ alleles is consistent with the observations that fetal γ/δ thymocytes frequently contain rearranged TCR β genes (25) and that γ/δ TCR-bearing thymocytes typically contain rearranged TCR β genes (26, 27). It is not known if such thymocytes form a special subset of γ/δ thymocytes or are precursors of α/β thymocytes.

Because the appearance of γ/δ thymocytes precedes the appearance of α/β thymocytes in ontogeny (28), the relationship of these thymocyte types is a matter of interest. Two indirect lines of evidence suggest that γ/δ thymocytes are not precursers of α/β thymocytes. First, it has been reported that a putative recombination signal required for TCR α gene rearrangement lies 3′ to the V_δ genes (29). This recombination signal would be deleted by V_δ-to-D_δ rearrangement, thereby preventing subsequent TCR α gene rearrangements (29, 30). Further, analysis of nuclear extrachromosomal DNAs formed from the excision of TCR δ genes during V_α-to-J_α joining has shown that the excised $D_{\delta 2}$ and $J_{\delta 1}$ genes are in germ-line configuration (31), suggesting that TCR α gene rearrangement occurs on chromosomes that have germ-line configuration TCR δ genes. Together, these findings imply that *V*-to-*D*–*J* rearrangement of TCR δ genes precludes TCR α gene rearrangement. However, analysis of cytoplasmic circular DNAs from α/β T cells has shown that some of these DNAs contain nonfunctional rearranged TCR δ genes, suggesting that rearrangement of TCR α genes can occur on chromosomes that have rearranged TCR δ genes (32). Thus, the developmental relationship of γ/δ thymocytes to α/β thymocytes remains ambiguous.

Because γ/δ thymocytes compose only 0.2% (23) of the potential thymocyte target cell pool for Ab-MuLV, our recovery of Ab-MuLV thymomas of the γ/δ thymocyte phenotype was unexpected. It may be that α/β thymocytes are not transformed by Ab-MuLV because they are not susceptible to Ab-MuLV infection or because the v-*abl* gene is expressed inefficiently in them. However, it has been shown that >90% of normal thymocytes could be infected by Mo-MuLV and express Mo-MuLV-encoded proteins (33). Because both Ab-MuLV(Mo-MuLV) and Ab-MuLV(ψ-2) are enveloped in Mu-MuLV *env* proteins, which determine the virus' host-cell range, and because the Ab-MuLV long terminal repeat (LTR) is virtually identical to the parent Mo-MuLV LTR (34), it is unlikely that Ab-MuLV fails to transform α/β thymocytes because of blocks to either Ab-MuLV infection or expression. Because the major difference between the parent Mo-MuLV and Ab-MuLV is the v-*abl* gene of Ab-MuLV, shown to be the sole viral determinant of Ab-MuLV pre-B-cell tropism (34), it is also likely that v-*abl* is the viral determinant of the observed Ab-MuLV γ/β thymocyte tropism. Thus, α/β thymocytes may not be transformed by Ab-MuLV because they do not produce the v-*abl* substrates necessary for Ab-MuLV transformation or because the v-abl protein is toxic to these cells.

Although Ab-MuLV has been reported to transform early thymocytes (9, 10), whether these transformants represented α/β or γ/δ thymocytes was unclear. We have shown that Ab-MuLV thymomagenesis occurs by the transformation of early γ/δ thymocytes. Just as studies of Ab-MuLV B-cell lines have provided much information on the processes of Ig gene rearrangement and B-cell differentiation, studies of Ab-MuLV thymoma cell lines may provide information on the mechanisms of TCR γ and δ gene rearrangement and on γ/δ thymocyte development.

G.D.H., K.I., D.A.K., and S.T. dedicate this paper to the memory of Rex Risser, who died unexpectedly on September 27, 1990. Rex will be sorely missed by all who knew him, both as a colleague and a good friend. We thank John McKearn for FACS analysis, and Jacqueline Arbuckle for excellent assistance. This work was supported in part by Public Health Service Grants CA-41302 to R.R. and CA-28900 and AI-17879 to S.T., Predoctoral Training Grant T32-CA-09135 to G.D.H., American Cancer Society Grant IM-439G to S.T., and a grant from the Ajinomoto Co.

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