THYMUS-LEUKEMIA (TL) ANTIGENS OF THE MOUSE

Analysis of TL mRNA and TL cDNA from TL^+ and TL^- Strains

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Thymus-leukemia (TL)[1] antigens were initially identified during a study of radiation-induced leukemias of the mouse (1). Subsequent studies revealed that the TL system had several unusual features (2). In certain strains, such as C57BL/6, TL antigens appeared to be restricted to leukemia cells and could not be detected on any other cell type, including thymocytes. In other strains, such as A strain mice, TL antigen expression was not limited to leukemia cells but could also be detected on normal thymocytes. A number of other mouse strains were tested, and strains could be classified as TL^+ or TL^- based on expression vs. nonexpression of TL in normal thymocytes. Anomalous TL expression in leukemia cells was also found in other TL^- strains. It appeared, therefore, that structural genes for TL are universal in the mouse, and that expression of TL is normally restricted to mice of TL^+ strains. Leukemogenesis disrupts this normal regulation of TL antigens in TL^- strains and brings about an activation or derepression of normally silent TL genes.

TL antigens are encoded by the *Tla* region on chromosome 17, less than two units to the right of the *H-2D* locus of the major histocompatibility complex (3). TL antigens belong to the class I antigen family, being comprised of 45–48,000 mol wt glycoproteins noncovalently bound to β_2-microglobulin (4–6). TL antigens differ from H-2 antigens, however, in that they do not serve as transplantation antigens and are much less polymorphic than H-2 antigens. Seven TL antigenic specificities (TL1 through TL7) have been described (2, 7, 8), and these classify inbred mice into six *Tla* haplotypes (Tla^a through Tla^f).

Tla region genes have recently been cloned from BALB/c (9, 10) and C57BL/10 (11). Transfection assays showed that several of the BALB/c *Tla* region clones code for TL antigens (10). Obata et al. (12) have generated *Tla* region–specific probes from C6.3, a TL-coding cosmid clone from BALB/c mice. One of these probes, pTL1, shows *Tla* region specificity (defined by restriction polymorphism with *Tla/Qa* region congenic strains) and TL specificity (defined by RNA transcripts in TL^+ and TL^- normal and leukemic cells). Using this probe, a genomic clone, *C25.1*, was isolated from ERLD (a TL^+ leukemia of TL^- strain C57BL/6) and found to express TL1 and TL2 in transfection assays. Sequence

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[1] *Abbreviations used in this paper:* SDS, sodium dodecyl sulfate; TL, thymus-leukemia.

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analysis of *C25.1* showed that the structure of this TL gene is clearly related to other class I genes. However, exons coding for the cytoplasmic domain are distinctly different in TL and H-2. In TL, this region is encoded by a single exon, whereas in H-2 it is the product of three exons (12).

In the present study we have analyzed the pattern of TL mRNA in normal and leukemic cells of TL^+ and TL^- mice. A cDNA library was constructed from ASL1 (a TL^+ leukemia of Tla^a mouse origin), and a number of TL cDNA clones were isolated and sequenced. The results indicate that TL genes from TL^+ and TL^- strains are highly conserved and that three or more structurally distinct genes are expressed in the A strain leukemia.

Materials and Methods

Mice and Leukemias. Mice were obtained from our breeding colonies at Memorial Sloan-Kettering Cancer Center. Origin and TL1, 2, 3, and 4 phenotypes of the leukemias are summarized in Table I.

Enzymes. Restriction enzymes, T4 ligase, and DNA polymerase I Klenow fragment were purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, MD), New England Biolabs, Inc. (Beverly, MA), or Boehringer Mannheim Biochemicals (Indianapolis, IN). Reverse transcriptase was from Life Sciences, Inc. (St. Petersburg, FL). S1 nuclease and terminal transferase were from Pharmacia, Inc. (Piscataway, NJ).

RNA Purification and Northern Blotting Analysis. Total cellular RNA was purified by the guanidinium/cesium chloride method (13). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose (Pharmacia, Inc.) column chromatography. Nuclear and cytoplasmic RNA was prepared as described by Maniatis et al. (14). RNA was fractionated in the presence of 2.2 M formaldehyde by agarose gel electrophoresis and transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, NH) as described (14). Blots were analyzed by hybridization with [^{32}P]dCTP (New England Nuclear, Boston, MA)-labeled, nick-translated probes (15) in the presence of 50% formamide for at least 24 h at 37°C. Filters were then washed three times in 2× standard saline citrate (SSC) with 0.2% sodium dodecyl sulfate (SDS) for 15 min each at 60°C, followed by two washes in 0.2× SSC with 0.2% SDS for 15 min each. X-ray films (XAR-5; Eastman Kodak Co., Rochester, NY) were exposed with or without an intensifying screen (Cronex Plus; DuPont Co., Wilmington, DE).

Construction and Screening of the cDNA Library. A cDNA library was constructed from poly(A)$^+$ RNA using dG-tailed PstI-digested pBR322 (Bethesda Research Laboratories, Inc.) according to the method of Villa-Komaroff et al. (16). Double-stranded cDNA was size-fractionated using a 5% polyacrylamide gel, and species of >250 basepairs (bp) were isolated and annealed to the vector. Transformation of *Escherichia coli* LE392 was carried

TABLE I
Characteristics of TL^+ and TL^- Leukemias

Designation	Strain of origin	*Tla* haplotype	TL specificity
ASL1	A	a	1, 2, 3
RADA1	A	a	1, 2, 3
ERLD	C57BL/6 (B6)	b	1, 2, 4
EL4	C57BL	b	—
AKM1	AKR	b	1, 2, 4
AK2A	AKR	b	—
RL♀3	BALB/c	c	1, 2, 4
RL♂1	BALB/c	c	1, 2
RVC	BALB/c	c	—

out by calcium chloride procedure (17) and ~200,000 colonies were obtained. LE392 transformants were plated or transferred to nitrocellulose filters (HATF; Millipore Corp., Bedford, MA), cultured overnight, lysed, and processed as described (18). Colony hybridization was done using published procedures (19) with nick-translated probes. Washing conditions were as described in Northern blotting analysis, except that the temperature was 42°C instead of 60°C.

DNA Sequencing and Analysis. DNA sequencing was carried out using the dideoxynucleotide chain terminator method of Sanger et al. (20). Restriction sites used for M13 subcloning are indicated in Fig. 3. PstI, SstI, and SmaI sites of M13mp18 and M13mp19 (21) were used. In the case of unidirectional cloning, M13 was restricted with PstI/SstI or PstI/SmaI to achieve appropriate cloning sites. DNA and protein sequence analysis and hydropathicity plots were performed using computer programs of IntelliGenetics Inc. (Palo Alto, CA).

Results

TL mRNA in Normal and Leukemic Cells of Different Tla Haplotypes. pTL1, a PvuII-PstI 600 bp fragment derived from a TL-coding gene of BALB/c mice, has been shown (12) to have TL specificity as determined in dot-blot analysis of RNA from a range of TL+ and TL- cell types. Fig. 1 shows Northern blot hybridization with pTL1 and poly(A)+ RNA from thymocytes and leukemic cells of mice of different *Tla* haplotypes. TL mRNA was found in all cells known to express TL antigen, but not in any cells having a TL- phenotype. In normal thymocytes of TL-expressing strains ($Tla^{a,c,d,e,f}$), multiple RNA transcripts were observed, ranging from 1.3 to 3.5 kb, and each haplotype could be distinguished on the basis of a distinctive pattern of mRNA species. TL+ leukemias of Tla^a and

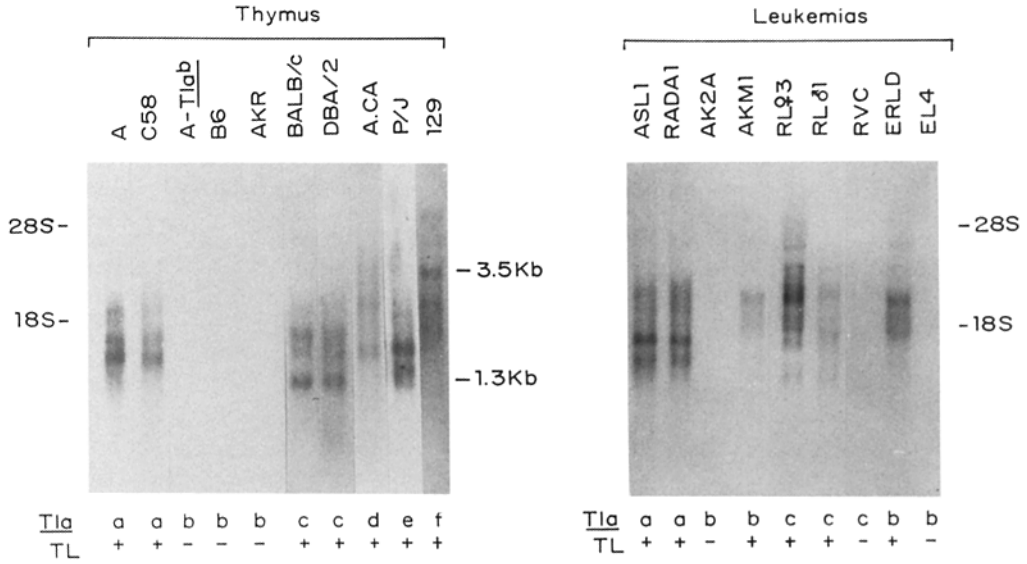


FIGURE 1. Northern blot hybridization of ^{32}P-labeled pTL1 with poly(A)+ RNA from different TL haplotypes. Poly(A)+ RNA was prepared from thymus and leukemias and analyzed with the TL-specific probe, pTL1. RL♀3 showed a major band at ~2.5 kb that is absent in cytoplasmic RNA and might be a nuclear precursor. The amount of RNA loaded per lane was adjusted for pattern comparison between different haplotypes and ranged from 1 to 8 μg. Autoradiography exposure time was 24 h, with an intensifying screen.

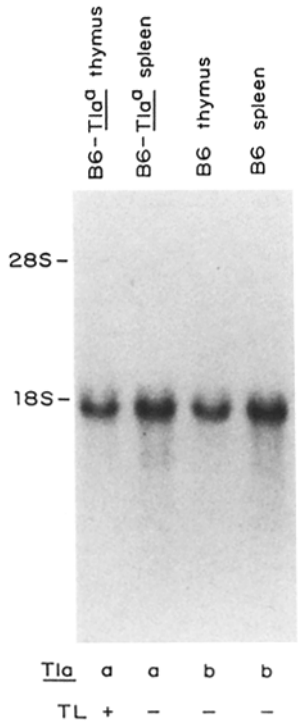


FIGURE 2. Northern blot hybridization of ^{32}P-labeled pH-2IIa with thymus and spleen total RNA. Total RNA was extracted from thymus and spleen of B6 and B6-*Tla*^{a} mice. The probe used was pH-2IIa, a class I cDNA probe extending from the end of exon 3 to part of the 3′ untranslated region. 15 μg of total RNA was loaded per lane, and exposure time was 4 h, with an intensifying screen.

Tla^c also have multiple bands and the same general patterns as normal thymocytes, although the intensity of individual bands showed some differences between normal and leukemic cells. *Tla*^{b} mice do not normally express TL antigens, and no TL mRNA could be found in *Tla*^{b} thymocytes. TL antigens are found in leukemias of *Tla*^{b} mice, and Fig. 1 shows TL mRNA in TL$^+$ leukemias of two *Tla*^{b} strains, AKR and B6. The pattern of mRNA in these TL$^+$ leukemias of *Tla*^{b} mice is similar, and can be distinguished from other *Tla* haplotypes. To determine whether nuclear precursors contribute to the size heterogeneity of TL mRNA, nuclear and cytoplasmic poly(A)$^+$ RNA were prepared and analyzed, and the results indicated that all major species are present in the cytoplasm as mature messages. mRNA preparations were also analyzed with pH-2IIa, a class I–specific probe (22) (Fig. 2). In contrast to the size heterogeneity of TL mRNA, the class I transcripts detected by pH-2IIa showed a uniform 1.6 kb band, known to be the size of mature H-2 transcripts (23).

Isolation and Characterization of pTL$^+$ cDNA Clones from a Leukemia of Tla^a Haplotype. A cDNA library was constructed from cytoplasmic poly(A)$^+$ RNA of ASL1 (a TL$^+$ leukemia of A strain), and 12 pTL1$^+$ clones were isolated. Restriction mapping and sequence analysis revealed that these clones are derived from at least three different genes, designated *Tla*^{a}*-1*, *Tla*^{a}*-2*, and *Tla*^{a}*-3* (Fig. 3).

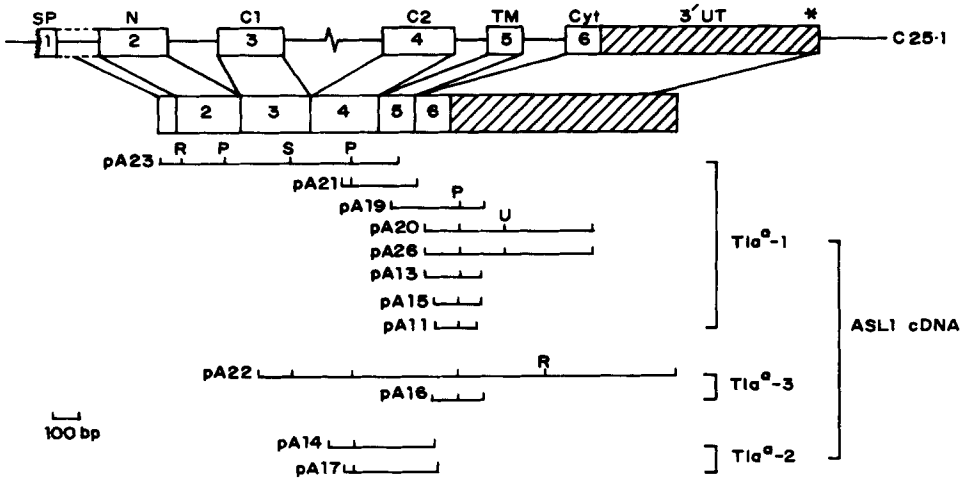


FIGURE 3. Exon structure and partial restriction map of TL cDNA clones. pTL1+ cDNA clones were isolated from ASL1 and sequenced using restriction sites shown (P, PstI; R, RsaI; S, SstI; U, PvuII). The sequences were compared with *C25.1* to assign exon boundaries, and define the position of each clone. Corresponding protein domains are shown on top of exons in *C25.1* (SP, signal peptide; TM, transmembrane domain; Cyt, cytoplasmic domain; 3'UT, 3' untranslated region), and the asterisk indicates the first poly(A) signal (AATAAA) in *C25.1*. The dashed lines between exon 1 and 2 denotes possible alternative splicing of intron 1. The TL cDNA clones were found to be derived from at least three genes in ASL1 (*Tla^a-1, 2, 3*) by sequence comparisons. (See text).

Eight clones (pA11, 13, 15, 19, 20, 21, 23, 26) are derived from *Tla^a-1*, two (pA14, pA17) from *Tla^a-2*, and two (pA22, pA16) from *Tla^a-3*.

The nucleotide and deduced amino acid sequences of these TL cDNA clones are presented in Fig. 4. The sequence of *C25.1* (a *Tla^b* genomic TL gene) (12) and the *H-2K^b* sequence (24) are shown for comparison. The TL cDNA clones differ from one another by multibase substitutions randomly distributed throughout the entire sequence, and no hypervariable clusters could be found. By comparing these TL cDNA sequences to *C25.1*, the exon-intron structure of *C25.1* was determined; the TL cDNA clones shown in Fig. 3 span the TL gene product from the second exon to the 3' untranslated region of exon 6. One of the ASL1 clones, pA23, has a 66 bp sequence corresponding to intron 1 of *C25.1*, and represents either a splicing intermediate or a product of alternative splicing.

A striking feature of these TL cDNA clones is their high degree of structural homology (Table II). *Tla^a-1* and *Tla^a-2* share 99% homology, with only 7 bp differing of the 463 compared. *Tla^a-3* is more distantly related, but still shows 94–95% sequence homology with either *Tla^a-1* or *Tla^a-2*. In comparing the sequence of *C25.1* (*Tla^b* haplotype) and *TLa^a* cDNA clones, we found >94% homology. Partial sequence (exons 4, 5, and 6) of a BALB/c (*Tla^c*) TL-coding gene, designated *R2*, has also been obtained (12 and unpublished results). *R2* showed >96% homology with *C25.1* and with *Tla^a* cDNA in the sequences compared.

Table III compares the structural homology of TL and H-2 genes, based on the sequences shown in Fig. 4 and the published sequence of H-2D^b (25) and H-

TABLE II
Percent Homology of TL Genes from Tla^a, Tla^b, and Tla^c Haplotypes

Tla haplotype:		*Tla^a*			*Tla^b*	*Tla^c*
	TL gene:	*Tla^a-1*	*Tla^a-2*	*Tla^a-3*	*C25.1*	*R2**
Tla^a	*Tla^a-1*		99 (463)	94 (1,273)	95 (1,641)	97 (554)
	Tla^a-2			95 (463)	97 (463)	98 (554)
	Tla^a-3				94 (1,698)	96 (554)
Tla^b	*C25.1*					96 (554)
Tla^c	*R2*					

Comparisons are based on overlapping regions between pairs, and the number of basepairs compared is shown in parenthesis. One deletion is considered as a single change regardless of the size.

* *R2*, a TL-coding gene of BALB/c (*Tla^c*) origin, was partially sequenced (exon 4, 5, and part of 6).

TABLE III
Percent Homology of Tla^a, Tla^b, and H-2 Genes

	Tla^a vs. *Tla^b*		*Tla^b* vs. *H-2K^b*		*H-2D^b* vs. *H-2K^d*	
	Nucleotide	Amino acid	Nucleotide	Amino acid	Nucleotide	Amino acid
Exon 2	93	90	73	56	93*	86
Exon 3	95	94	76	64	89	78
Exon 4	95	93	91	89	96	90
Exon 5	96	92	45‡	19‡	85	75
Exon 6§	98	96	—	—	100	100
Exon 7					90	69
Exon 8						

Tla^a sequence is from *Tla^a-1*, and *Tla^b* sequence is from *C25.1*. A deletion is considered as one change regardless of the length.

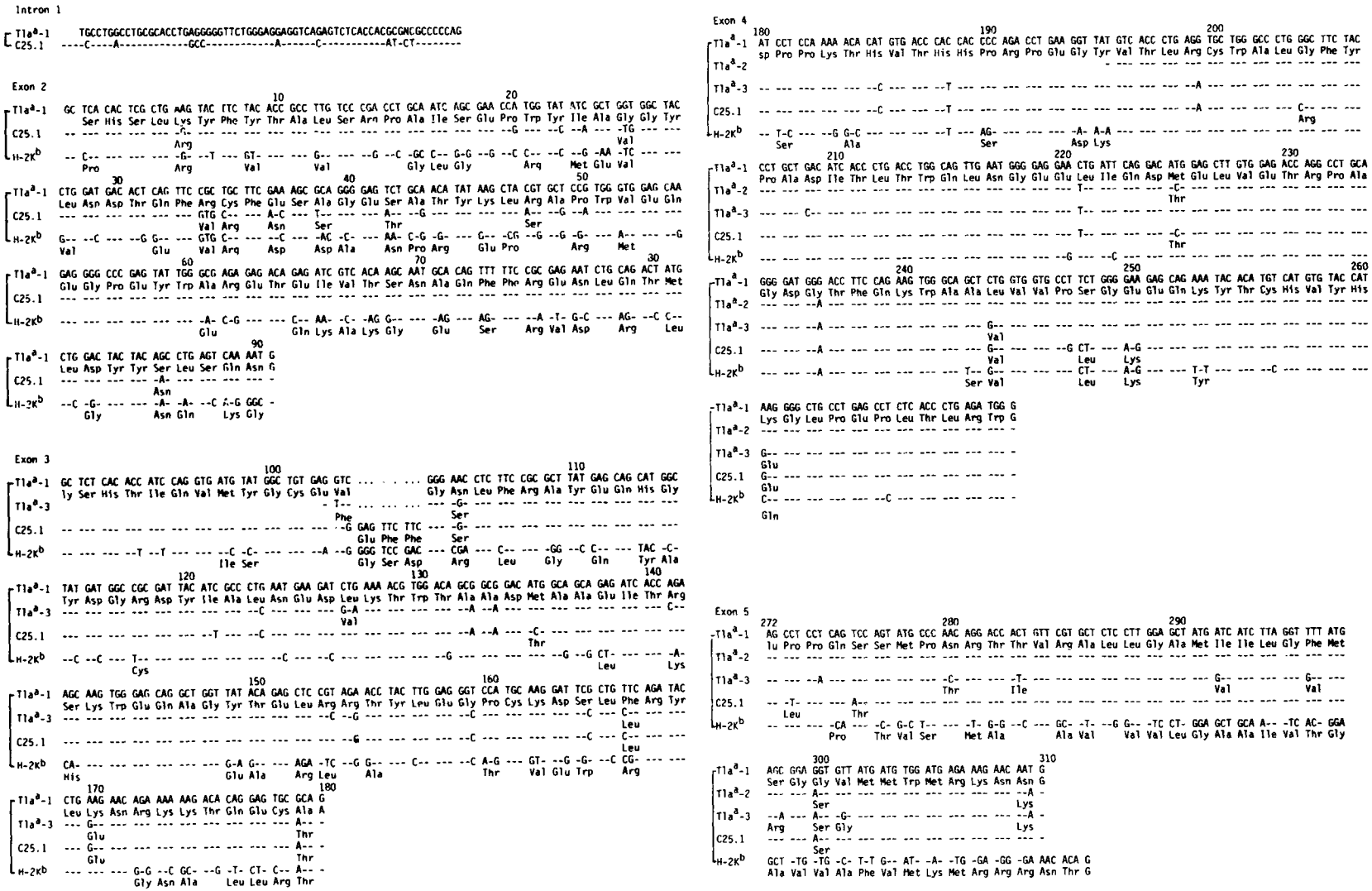
* Only 30 bp are available in *H-2D^b* for comparison.

‡ No gaps were generated.

§ Only the amino acid coding region is compared.

2K^b (24). Different *Tla* haplotypes showed >90% homology in all exons compared. Homology between H-2 and TL ranged from >90% in exon 4 (β_2-microglobulin-binding domain) to <50% in exon 5 (transmembrane domain). As discussed below, the exons coding for the cytoplasmic domain of TL and H-2 have completely different structures.

Characteristics of 3′ Untranslated Region of TL mRNA. Sequence analysis of TL cDNA clones and *C25.1* revealed that multiple polyadenylation signals exist in the 3′ untranslated region of TL exon 6, and that this can account for some of the size heterogeneity of TL mRNA (Fig. 5). For example, both pA26 and pA19 appear to be derived from *Tla^a-1*. However, pA19 has only 155 bp of 3′ untranslated region, with the poly(A) tract found 12 bp downstream from a variant polyadenylation signal, ATTAAA. In contrast, this signal is not used in pA26. In the case of pA22, which is derived from *Tla^a-3*, a single base change from A to T results in the loss of the poly(A) signal used by pA19. However, there is a second polyadenylation signal (AATAAA) 720 bp downstream in


Intron 1
Exon 2
Exon 3
Exon 4
Exon 5

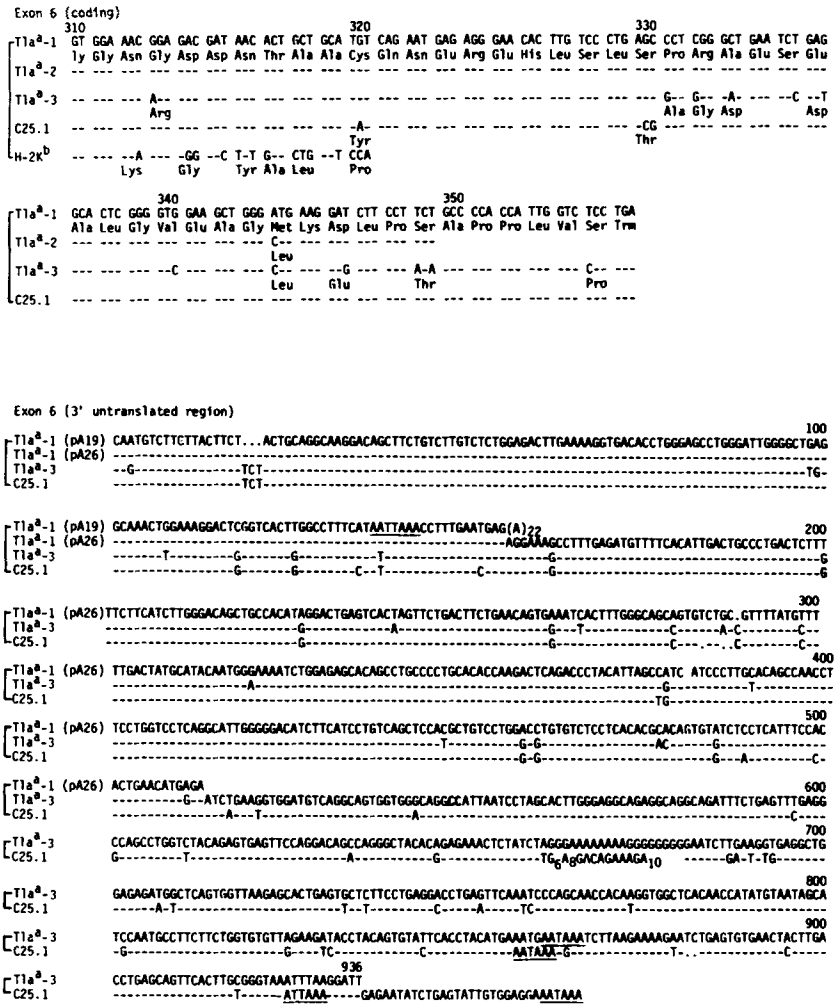


FIGURE 4. DNA sequences and predicted amino acid sequences of TL cDNA clones. TL cDNA sequences were derived from sequencing overlapping clones shown in Fig. 3, and compared to *C25.1* and H-2K^b. Amino acid numbers are shown above the sequences. Dashes indicate identity to the top sequence, and deletions are shown as dots.

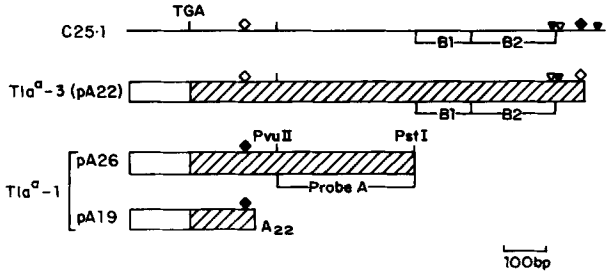


FIGURE 5. Potential polyadenylation sites at the 3′ end of TL genes. TGA is the termination codon of the clones; hatched areas indicate 3′ untranslated regions of the cDNA clones. B1 and B2 stand for types 1 and 2 Alu-like repetitive sequences (26). Potential polyadenylation signals are shown: (▲) AATAAA and (♦) ATTAAA. (△) AATGAA and (◇) ATTTAA have not been shown to serve as polyadenylation signals. Probe A is the probe used in Fig. 6.

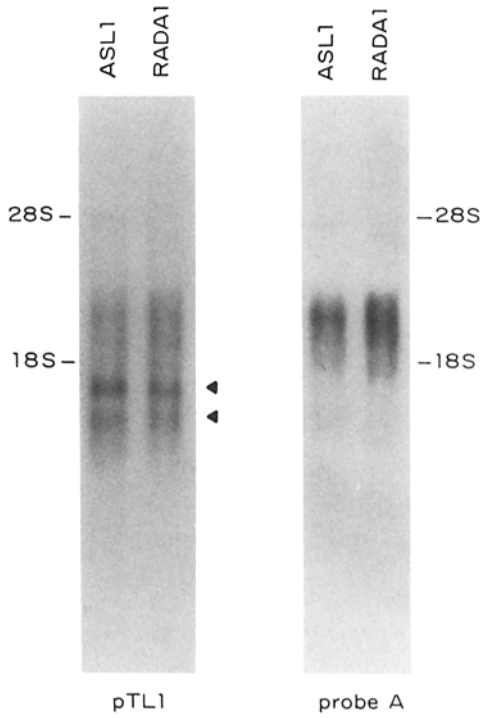


FIGURE 6. Northern blot analysis of ASL1 and RADA1 poly(A)+ RNA with different probes. ASL1 and RADA1 poly(A)+ RNA were prepared and analyzed with either pTL1 or probe A (see Fig. 5). Arrowheads indicate 1.4 and 1.6 kb bands that do not hybridize to probe A.

pA22. This does not seem to be used, because pA22 ends 70 bp beyond the poly(A) signal, without a poly(A) tract. Two additional polyadenylation signals were found in *C25.1* within a 200 bp stretch immediately 3′ to the type 2 Alu-like sequences. Evidence that TL mRNA heterogeneity can result from different polyadenylation sites is shown in Fig. 6. ASL1 and RADA1 mRNA were analyzed by Northern blot analysis with pTL1 and a PvuII-PstI fragment derived from pA26 (probe A). This probe contains a 300 bp stretch following the first polyadenylation signal in *Tla^a-1*. With pTL1, at least three species of TL mRNA

were seen: 1.4 kb, 1.6 kb, and a high molecular weight smear at ~2.1 kb. With probe A, only the 2.1 kb smear was evident. Analysis of TL mRNA from BALB/c thymus and leukemias showed similar results.

Primary Structure of TL Antigens as Deduced from cDNA Sequences. Assuming that pA23 (*Tla^a-1*) is a splicing intermediate, the TL cDNA sequences presented in Fig. 4 would code for a polypeptide 355 amino acids long, with an unglycosylated molecular weight of ~40,000. This primary structure of mature TL antigen has the following features. Exon 2 (amino acid position 1–90) codes for the N domain of TL antigen. This domain contains an N-glycosylation site in H-2 (position 86 in H-2K^b) that is not found in *Tla^a-1*. A potential glycosylation site in *Tla^a-1* (Asn-X-Ser) occurs in position 90. Exon 3 (position 91–179) codes for the C1 domain. The corresponding region in *C25.1* and H-2 has 92 amino acids, compared with 89 amino acids in the TL cDNA products. This is due to a 9 bp deletion in all TL cDNA clones starting at position 103. In this exon, there are two cysteine residues (positions 101 and 161) that can form the interchain disulfide bond in the C1 domain. No glycosylation sites are found in this exon, distinguishing it from H-2, in which an N-glycosylation site is found in position 176 of H-2K^b. Exon 4 (position 180–271) codes for the β_2-microglobulin-binding domain, C2. A second disulfide bond can be formed between cysteine residues in positions 200 and 256. Exon 5 (position 272–309) codes for the transmembrane domain. Hydropathicity analysis indicates that an extremely hydrophobic stretch of 20 uncharged amino acids (position 286–305) is encoded within this exon. Two to three positively charged residues were found between positions 305 and 309, and these may interact with membrane phospholipids and serve in membrane anchorage. Another potential glycosylation site coded for by exon 5 is found in *Tla^a-1* and *Tla^a-2* (position 280). This site is not conserved in *Tla^a-3*. As it is very near the hydrophobic stretch, we suspect that it is not used for glycosylation. Exon 6 (position 310–355) codes for the cytoplasmic domain and the 3′ untranslated region of TL. Like H-2, this domain has a high content of polar amino acids (60%). Serine and threonine residues found in this domain might serve as sites of phosphorylation.

Discussion

With the development of genetic probes to detect TL genes and TL mRNA, some fundamental issues concerning the TL system can be clarified. Answers to questions such as the number and structure of TL genes in various mouse strains and the regulation of TL expression in (*a*) thymocytes of TL$^+$ and TL$^-$ strains, (*b*) T lymphocytes of TL$^+$ strains, and (*c*) TL$^+$ leukemia occurring in TL$^-$ strains, require approaches beyond conventional serology and biochemistry. This report and that of Obata et al. (12) provide information on the structure of TL genes and TL mRNA in mice of *Tla^a*, *Tla^b*, *Tla^c* haplotypes. Restriction fragment analysis with pTL1, a TL-specific probe, indicates that mouse strains differ in the number of TL genes carried, with TL-expressing strains having ≥4 (*Tla^{a,c,e}*), ≥3 (*Tla^d*), or ≥2 (*Tla^f*) TL genes, and non–TL-expressing strains (*Tla^b*) having ≥2 TL genes. To determine how many TL genes are expressed we constructed a cDNA library from a TL$^+$ leukemia of A strain (*Tla^a*) origin. Sequencing of pTL1$^+$ cDNA clones indicated that at least three TL genes are expressed in the

A strain leukemia. Sequence comparisons of these *Tla*^{a} cDNA clones and TL-coding genes of *Tla*^{b} and *Tla*^{c} origin indicate that the structure of TL genes has been highly conserved. Equally strong homology is seen in TL from the same or different haplotypes. This low level of polymorphism at the genetic level is consistent with conclusions drawn from serological study of TL antigens, and contrasts with the highly polymorphic nature of other class I genes, such as *H-2D* and *H-2K*. The *Qa2,3* locus, located on chromosome 17 between *H-2D* and *Tla*, also codes for class I antigens (27). *Qa2,3* region genes (gene 27.1 [28] and Q10 [29]) have been sequenced, and their structure is clearly homologous to H-2. However, Q10 alleles show little polymorphism and, in this regard, more closely resemble TL. A number of mechanisms have been postulated to account for H-2 polymorphism, including gene conversion, gene duplication and deletion, and high recombinational frequencies (30). Accumulating evidence points to gene conversion as the major cause of polymorphism at the *H-2K* locus (24, 31). As suggested by Mellor et al. (29) to account for the low level of polymorphism in Q10 genes, TL genes may also be unable to serve as recipients of gene conversion between class I genes. Alternatively, TL may engage in gene conversion events, but then be lost due to selective pressure. As TL antigens apparently are never expressed in normal *Tla*^{b} mice, there is no obvious selective pressure to preserve their structure and prevent genetic drift. The finding that TL genes of *Tla*^{a}, *Tla*^{b}, and *Tla*^{c} mice are highly conserved argues that there are in fact selective pressures acting on TL genes and that the low degree of TL polymorphism in mice is a reflection of this. A large number of other class I genes are present in the *Tla* region (11, 32), and it will be important to know whether these also show a low level of structural polymorphism.

Although the general exon/intron organization of H-2 and TL is similar and clearly related, the exon 6 region of TL is distinctly different from that of other class I genes. In H-2, three exons (exons 6, 7, and 8) code for the cytoplasmic domain, which is followed by a 400–500 bp 3′ untranslated region. In TL, the entire cytoplasmic domain is encoded by a single exon (exon 6). The 3′ untranslated region of TL is of variable length (155 to >930 bp) due, at least in part, to the use of alternative polyadenylation signals. Types 1 and 2 rodent Alu-like repetitive sequences are found juxtaposed in the 3′ untranslated region of TL; due to the use of different polyadenylation signals, these repetitive sequences may or may not be found in the mature message. Type 1 Alu-like repetitive sequences have not been described in the 3′ end of other class I genes, whereas type 2 Alu-like sequences are found in L^d and D^b, but not in K^d transcripts (30). Kress et al. (33) have suggested that type 2 Alu-like sequences may be involved in the regulation of D and L antigen expression and account for the fact that K antigens are expressed to a higher level than D or L antigens. A role for type 1 and 2 Alu-like sequences in the differential expression of TL antigens is a possibility worth investigating. In addition, the distinctive structure of the cytoplasmic domain of TL may be important with regard to another feature of TL antigens, their ability to undergo antigenic modulation after exposure to TL antibody (34).

Since TL genes share sequence homology with other class I genes, it might be expected that general probes for class I genes, such as the 3′ probe pH-2IIa,

would hybridize with TL mRNA, as it does with TL genes. As seen in Fig. 2, only one species of mRNA hybridized with pH-2IIa, correponding to the mature H-2 transcript. The low degree of structural homology between H-2 and TL in the 3′ region is one reason for the failure to visualize TL mRNA with pH-2IIa. Another reason is the quantitative difference between TL and H-2 messages. TL messages are relatively rare in comparison with H-2 messages and, even with a TL-specific probe, longer exposure is necessary to visualize TL mRNA than is required for H-2 mRNA.

Northern blot analysis with pTL1 revealed multiple mRNA species. Use of alternative polyadenylation signals in the 3′ untranslated region of TL has been shown to contribute to the size heterogeneity of TL mRNA observed in TL^+ cells. However, there appears to be more mRNA species than can be attributed to this fact alone. Differential splicing of TL messages could also contribute to size heterogeneity. Another possibility is that non–TL-coding genes in the *Tla* region share sufficient homology with TL genes for their messages to be detected in Northern blots by pTL1. If this were the case, these genes would be expressed only in TL^+ cells, since pTL1 shows TL specificity with regard to TL expression.

Regulation of TL expression in mice of the Tla^b haplotype (e.g., B6, AKR) is of particular interest. TL antigens cannot be detected in normal thymocytes of these mice, and immunization with TL^+ cells results in the formation of TL antibodies (in contrast to mice of TL^+ strains, where immunological tolerance of TL antigens is the rule) (1). Despite the TL^- phenotype of normal Tla^b thymocytes, T cell leukemias arising in these mice, particularly when x-ray induced, frequently express TL antigens. Although Michaelson et al. (35) have suggested that low levels of TL can be detected in normal B6 thymocytes by biochemical methods, we have found no evidence for TL mRNA in normal B6 thymocytes by Northern blot or RNA dot blot analysis using a range of RNA concentrations. In contrast, TL mRNA can be easily detected in TL^+ leukemias of Tla^b haplotype. Previous studies (12) have demonstrated at least two $pTL1^+$ genes in B6, and cDNA libraries from B6 TL^+ leukemias, will indicate how many B6 TL genes are transcriptionally active. In addition, structural comparison of TL genes and adjacent flanking sequences from normal and leukemic B6 cells may provide insight into the mechanism of TL activation during leukemogenesis.

Summary

A thymus-leukemia (TL)-specific probe, pTL1, has been generated from a TL-coding gene of BALB/c mice. Multiple species of TL mRNA were detected in TL^+ cells by Northern blot analysis with pTL1, and different *Tla* haplotypes could be distinguished on the basis of characteristic patterns of TL mRNA. No TL-related message was found in normal or leukemic TL^- cells, including thymocytes from Tla^b mice. However, TL mRNA could be detected in TL^+ leukemias occurring in Tla^b mice. A cDNA library has been made from ASL1 (a TL^+ leukemia of A mice [Tla^a]), and $pTL1^+$ clones have been sequenced. At least three structurally distinct TL genes are expressed in ASL1. Sequence comparison of TL genes from three *Tla* haplotypes indicates that TL genes are highly conserved (>90% homology) and are more distantly related to H-2 genes.

Several polyadenylation sites have been found in the 3′ untranslated region of TL genes, and differential polyadenylation contributes to the size heterogeneity of TL transcripts. The predicted amino acid sequence of TL products indicates that TL and H-2 are similar in domain structure and disulfide bonds, but differ in glycosylation sites and in cytoplasmic domain sequences.

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