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Immunology

Structural analysis of *TL* genes of the mouse

(class I genes/differentiation antigens/leukemogenesis)

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ABSTRACT **Three *Tla* region-specific probes have been generated from the BALB/c genomic cosmid clone C6.3. One probe, pTL1, corresponds to 3′ sequences of a thymus leukemia (TL)-encoding gene, whereas pTL2 and pTL3 detect noncoding flanking sequences. The TL specificity of pTL1 was demonstrated by studies of RNA from thymocytes of TL^+ and TL^- mouse strains and from TL^+ and TL^- leukemias; presence/absence of $pTL1^+$ transcripts correlated with presence/absence of TL antigens detected serologically. Nine *Tla* haplotypes were defined by restriction fragment polymorphism with pTL1, and the number of *TL* genes has been estimated to be ≥4 in Tla^a, Tla^c and Tla^e mice, ≥3 in Tla^d mice, and ≥2 in Tla^b and Tla^f mice. A TL-encoding gene (*C25.1*) from the C57BL/6 TL^+ leukemia ERLD has been cloned and sequenced, and the exon/intron organization of *C25.1* has been deduced from the structure of $pTL1^+$ cDNA clones and from the known organization of *H-2* genes. The major structural differences between *TL* and *H-2* genes are in exons coding for the cytoplasmic domain.**

TL (thymus leukemia) antigens are cell-surface differentiation antigens restricted to thymocytes in normal mice (1). Not all mouse strains express TL, permitting strains to be classified as TL^+ or TL^-. TL antigens are determined by the *Tla* locus, closely linked to the *H-2/Qa* loci, on chromosome 17. Six different *Tla* haplotypes can be distinguished (1–3). TL antigens resemble class I major histocompatibility complex antigens, being glycoproteins with the M_r 45,000 associated with β_2-microglobulin (4, 5). A striking feature of TL is the appearance of TL^+ leukemias in TL^- mice (6). This anomalous expression indicates that TL structural information is universal in the mouse and that regulatory genes control expression vs. nonexpression of TL antigens. The cloning of *Tla*-region genes by Steinmetz *et al.* (7) and Weiss *et al.* (8) allows detailed analysis of the structure and regulation of *TL* genes. In this study, we have generated and characterized a TL-specific probe and used it to ask questions about TL.

MATERIALS AND METHODS

Mice and Leukemias. Mice were obtained from our breeding colonies at Memorial Sloan–Kettering Cancer Center or from The Jackson Laboratory. The TL^+ and TL^- leukemia cells selected for study were derived from C57BL/6 (B6), A, AKR, and BALB/c mice.

Class I Probes and *Tla* Region Clones. Probes for class I genes, pH-2IIa and pH-2III, were derived by Steinmetz *et al.* (9). BALB/c λ17.3 and C6.3 (refs. 7 and 10) were provided by Leroy Hood (California Institute of Technology, Pasadena, CA) and B10 *Tla* region cosmids H11, H10, S14, LSK14, B2.7, and H6 (ref. 8) were provided by Richard A. Flavell and Karen Fahrner (Biogen Research, Cambridge, MA).

Transfection. Ltk^- cells in culture were co-transfected with DNA from genomic clones and the cloned HSV *tk* gene, using the calcium phosphate precipitation method (10, 11).

Serological Analysis. TL antigens were detected by cytotoxic/absorption assays and erythrocyte rosetting assays using conventional TL typing sera for TL specificities TL1, -2, -3, and -4 and mouse and rat monoclonal TL antibodies (1, 3).

Enzymes. Enzymes were purchased from Bethesda Research Laboratories, Boehringer Mannheim, New England Biolabs, or Life Sciences.

Preparation of DNA and RNA. Plasmid and cosmid DNA was isolated by the cleared lysate method (25), and mouse genomic DNA was prepared by the proteinase K-sodium dodecyl sulfate procedure (26). Total RNA was isolated from normal and leukemic tissues by using guanidine thiocyanate/CsCl (27), and poly(A)$^+$ RNA was prepared by oligo(dT)-cellulose chromatography (28).

Southern and RNA Dot Blot Analysis. DNA·DNA hybridization was performed according to the method of Southern (12) and RNA dot blot tests, following the method of Thomas (13).

Cosmid Library. A cosmid library was constructed using the pTL5 vector (14), following the method of Steinmetz *et al.* (7).

cDNA Library. cDNA libraries were constructed using dG-tailed pBR322 according to Villa-Komaroff *et al.* (15).

DNA Sequencing. DNA was sequenced by the method of Sanger *et al.* (16) using phage M13 mp18 or mp19 (17).

RESULTS AND DISCUSSION

***TL* Expression in L Cells Transfected with λ17.3.** λ17.3 is derived from BALB/c (Tla^c) mice. BALB/c thymocytes have a $TL1^-2^+3^-4^-$ phenotype, whereas BALB/c leukemias are $TL1^+2^+3^-4^-$ or $TL1^+2^+3^-4^+$. L cells transfected with λ17.3 expressed TL1 and -2 but not TL3 or -4. Mouse and rat monoclonal antibodies specific for TL antigens also reacted with λ17.3-transfected cells, confirming the results of Goodenow *et al.* (10). λ17.3 is known to contain two class I genes (10), and subcloning, transfection, and serological analysis have shown that one of them, R2, contained in a *Hin*dIII 8.7-kilobase (kb) fragment, codes for TL1 and -2.

Generation of a *TL*-Specific Probe from C6.3. C6.3 is a cosmid clone derived from BALB/c mice that contains the two class I genes of λ17.3 (7, 10). C6.3 was subcloned in pBR322 or pUC9 using a variety of restriction enzymes (Fig. 1). Three *Tla* region-specific probes were derived. pTL1 is a 600-base-pair (bp) *Pvu* II/*Pst* I fragment from the 3′ coding region of R2. pTL2 and pTL3 are 800-bp *Hin*dIII/*Pst* I and

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Abbreviations: TL, thymus leukemia; bp, base pair(s); kb, kilobase(s); B6, C57BL/6.

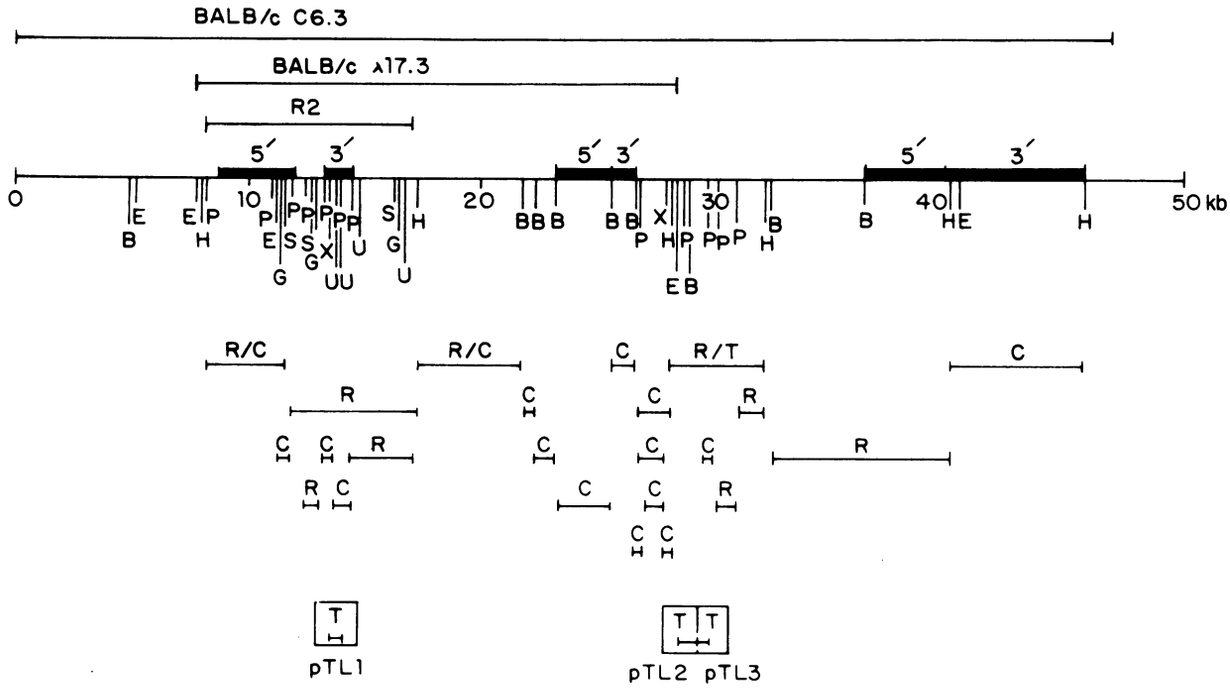

FIG. 1. Generation of *Tla*-region probes. BALB/c C6.3 was subcloned in pBR322 or pUC9 using a variety of restriction enzymes, and the resulting 26 clones were tested for restriction polymorphism by Southern blot analysis with DNA from *Tla/Qa* region congenic strains. Three hybridization patterns were observed: T, *Tla*-region specific; C, multiple bands with patterns similar to class I pH-2IIa and pH-2III probes; and R, smear, indicative of repetitive sequences. Restriction enzyme sites are identified as follows: B, *Bam*HI; E, *Eco*RI; G, *Bgl* II; H, *Hin*dIII; P, *Pst* I; S, *Sst* I; U, *Pvu* II; X, *Xba* I. Location and orientation of class I genes in C6.3, λ17.3, and R2 were determined by reactions with the pH-2IIa and pH-2III class I probes.

500-bp *Pst* I fragments from the 3′ flanking region of the second class I gene of C6.3 (Fig. 1). These probes showed *Tla*-region specificity (and low copy number) by restriction polymorphism analysis of DNA from *Tla/Qa* region congenic strains. Three patterns were observed by using DNA digested with restriction enzyme *Bam*HI, *Eco*RI, or *Hin*dIII. One related to the Tla^a haplotype (A, B6-Tla^a), one to the B6 Tla^b haplotype (B6, A-Tla^b), and one to the AKR Tla^b haplotype (AKR, B6.K1, B6.K2). Southern blot analysis of these probes with DNA digested by *Bam*HI is shown in Fig. 2. pTL1, pTL2, and pTL3 were also analyzed by RNA blotting with RNA from TL^+ cells; only pTL1 hybridized. In order to define the TL specificity of pTL1, RNA from the following TL^+ and TL^- cell types was tested: (*i*) thymocytes from 14 TL^+ and 11 TL^- mouse strains; (*ii*) thymocytes, spleen, liver, and kidney cells from strain A mice; and (*iii*) 13 TL^+ and 7

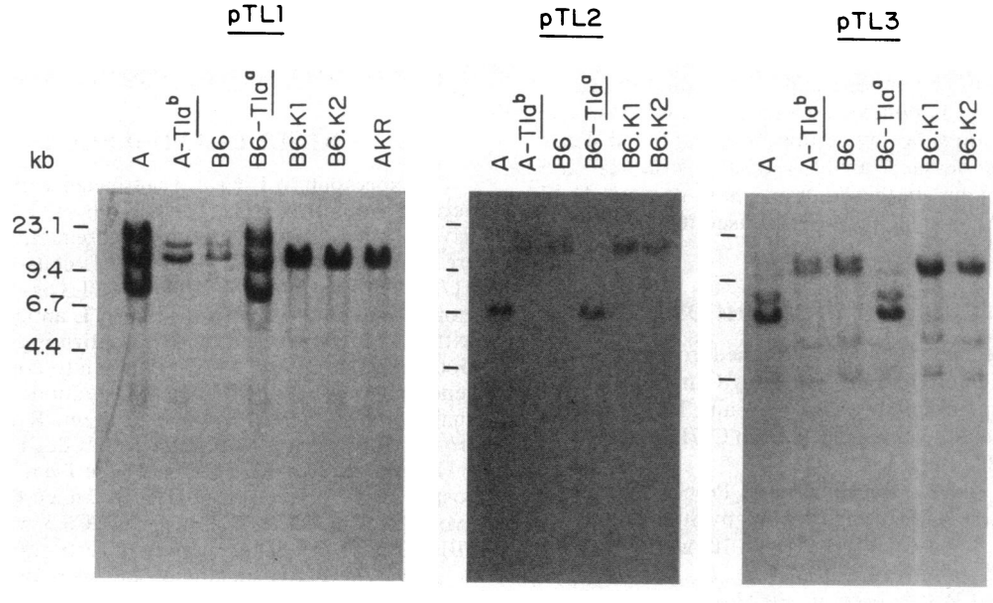

FIG. 2. *Tla* region specificity of pTL1, pTL2, and pTL3 by Southern blot analysis with DNA from *Tla/Qa* region congenic strains. Each lane contains 15 μg of DNA digested with *Bam*HI. Hybridization was performed at 65°C and filters were washed at 53°C. *H-2D/Qa-2/Tla* genotypes of the seven mouse strains are as follows: A, $H\text{-}2D^d/Qa\text{-}2^a/Tla^a$; A-$Tla^b$, $H\text{-}2D^d/Qa\text{-}2^a/Tla^b$ (Tla^b derived from B6); B6, $H\text{-}2D^b/Qa\text{-}2^a/Tla^b$; B6-$Tla^a$, $H\text{-}2D^b/Qa\text{-}2^a/Tla^a$ (Tla^a derived from A); B6.K1, $H\text{-}2D^b/Qa\text{-}2^b/Tla^b$ (Tla^b derived from AKR); B6.K2, $H\text{-}2D^b/Qa\text{-}2^a/Tla^b$ (Tla^b derived from AKR); AKR, $H\text{-}2D^k/Qa\text{-}2^b/Tla^b$.

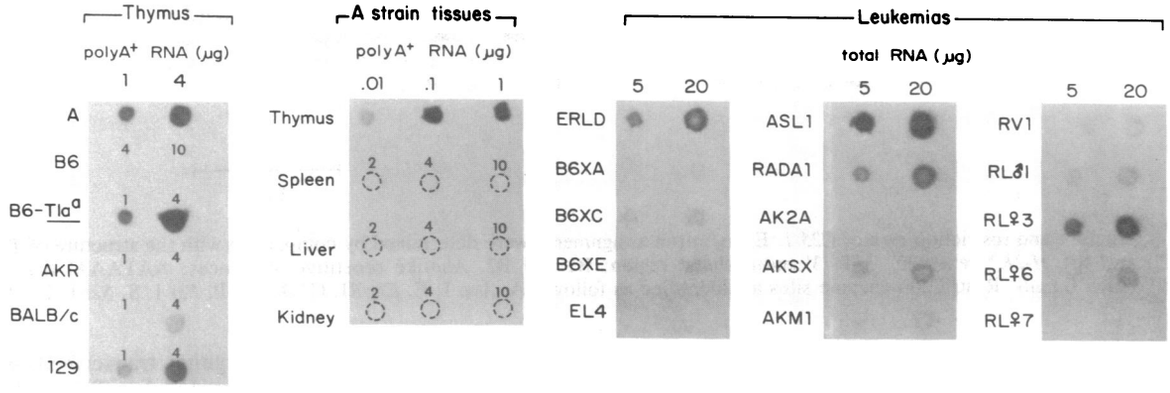

FIG. 3. Dot blot hybridization of RNA from TL$^+$ and TL$^-$ cells with pTL1. TL$^+$ thymuses: A, B6-*Tla*^{a}, BALB/c, and 129. TL$^-$ thymuses: B6 and AKR. Strain A TL$^+$ tissue: thymus; strain A TL$^-$ tissues: spleen, liver, and kidney. TL$^+$ leukemias: ASL1, RADA1 (strain A origin), ERLD, B6XA, B6XC, B6XE (B6 origin), AKSX, AKM1 (AKR origin), RV1, RL♂1, RL♀3, and RL♀6 (BALB/c origin). TL$^-$ leukemias: EL4 (C57BL origin), AK2A (AKR origin), and RL♀7 (BALB/c origin).

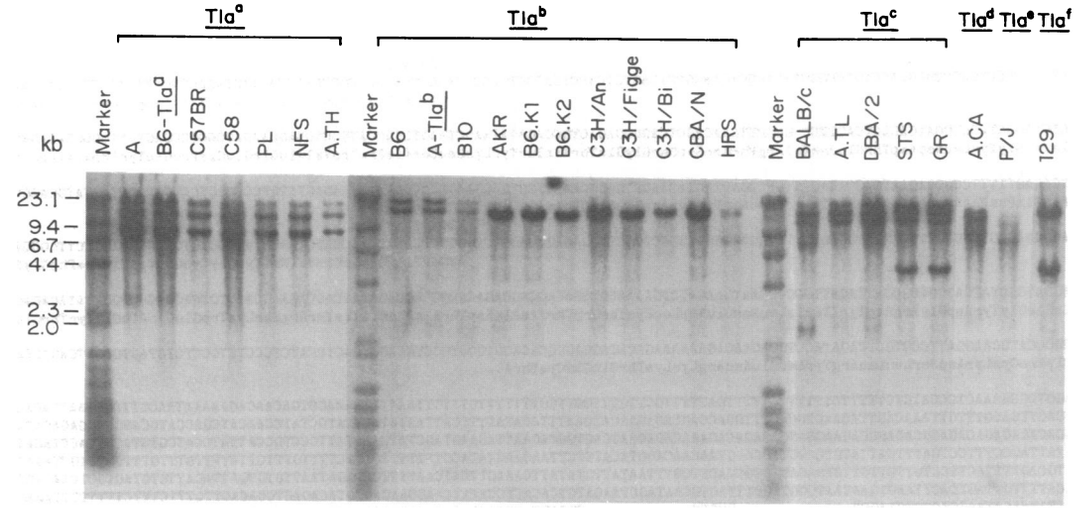

FIG. 4. Southern blot analysis of *Bam*HI-digested DNA from 26 mouse strains using the pTL1 probe. *Tla*^{a,b,c,d,e,f} refer to serologically defined haplotypes. Restriction fragment length polymorphism indicates nine distinct haplotypes.

TL$^-$ mouse leukemias. pTL1 detected RNA transcripts in all TL$^+$ cells but not in any TL$^-$ cell type. Examples of RNA dot blot tests are shown in Fig. 3. From these studies, we conclude that pTL1 has TL specificity.

How Many *Tla* Haplotypes? Six *Tla* haplotypes have been described by serological methods (1–3). Southern blot analysis using the pTL1 probe and *Bam*HI-digested DNA from 26 mouse strains indicated that at least nine *Tla* haplotypes could be identified (Fig. 4): one pattern for *Tla*^{a} (7 strains), three for *Tla*^{b} (prototypes B6, AKR, and HRS/J), two for *Tla*^{c} (prototypes BALB/c and STS/A), and one each for *Tla*^{d}, *Tla*^{e}, and *Tla*^{f}.

How Many *TL* Genes? Winoto *et al.* (18) assigned five clusters to the *Tla* region of BALB/c and Weiss *et al.* (8) assigned 13 genes to the *Tla* region of B10. However, the number of genes coding for TL structural information is not known. We estimate on the basis of the following data that there are at least 4 *TL* genes in *Tla*^{a}, *Tla*^{c}, and *Tla*^{e}; 3 in *Tla*^{d}; and 2 in *Tla*^{b} and *Tla*^{f} mice. Digestion of DNA by *Eco*RI yields four pTL1-hybridizing fragments in A (*Tla*^{a}) and BALB/c (*Tla*^{c}) and two in B6 (*Tla*^{b}). When the DNA of these mice was digested with *Pst* I, pTL1 hybridized to only one common 900-bp fragment, implying that this fragment is conserved in the *TL* genes of these haplotypes. Sequential digestion with *Eco*RI and *Pst* I also yields one 900-bp fragment (indicating no *Eco*RI site within the *Pst* I fragment) (Fig. 5). Therefore, each *Eco*RI fragment represents distinct *TL* genes. Similar results were obtained with the other mouse strains, with the exception of A.CA and 129 where an additional *Pst* I fragment, also not cut by *Eco*RI, was

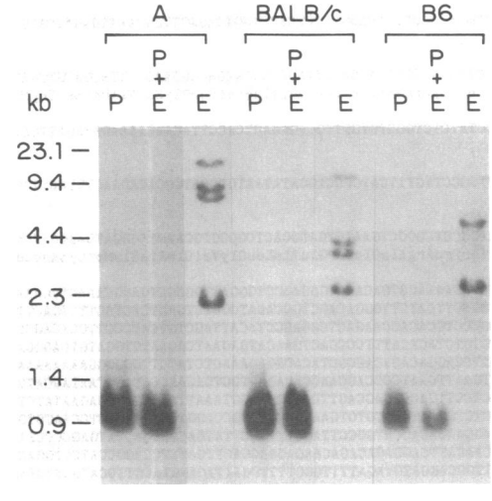

FIG. 5. Estimation of the number of *TL* genes in A, BALB/c, and C57BL/6 (B6) mice. DNA was digested with *Pst* I (P), *Eco*RI (E), or *Pst* I followed by *Eco*RI. Results indicate ≥4 *TL* genes in strain A and BALB/c mice and ≥2 *TL* genes in B6 mice.

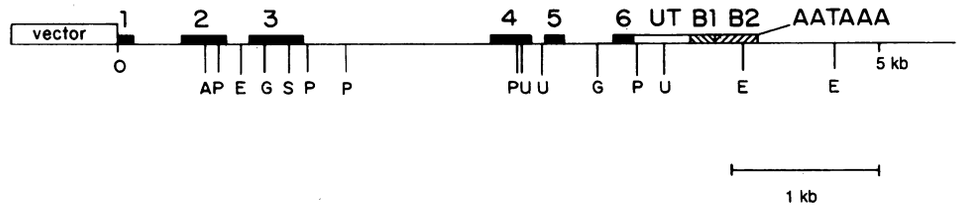

FIG. 6. Structure and restriction map of *C25.1*. Exon/intron assignments were determined by comparison with the structure of *TL* cDNA clones (19) and the *H-2K*^{b} gene (20). UT, 3′ untranslated region; B1 and B2, *Alu*-like repetitive sequences; AATAAA, first of three polyadenylylation signals. Restriction enzyme sites are identified as follows: A, *Ava* I; E, *Eco*RI; G, *Bgl* II; P, *Pst* I; S, *Sst* I; U, *Pvu* II.

observed. The conclusion that B6 has two *TL* genes is consistent with our finding of two pTL1$^+$ genes (*T1* and *T3*) in the B10 *Tla* region library of Weiss *et al.* (8).

How Many *TL* Genes Are Transcribed? As a first step in determining the number of *TL* genes transcribed, we have constructed cDNA libraries from ASL1 (a TL$^+$ leukemia of A strain) and from ERLD (a TL$^+$ leukemia of B6). Twelve pTL1$^+$ clones from ASL1 and six clones from ERLD were

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CLONE 25.1

    EXON 1
---GATCCTCCTGGCTGCCTCACAAGGCCAGACCCAGACCTGCCCAGGTGAGTGCTGGATGAGGAGGGAAACAGCCTTTTAGGGGAGGGGCGGAGGTTCCACCCTCCACCAGACCCTCCACCACTTCTCCACTCAAGGCC  140
    IleLeuLeuAlaAlaSerGlnGlyGlnThrGlnThrCysProG
                                                                                                                    EXON 2
TAGACCTGAGTCCTTCTACTTCCCTGACCTGCGCACCTGGCCGGGTTCTGGGAGAAGGTCACAGTCTCACCACATGCTGCCCCCAGGCTCACACTCGCTGAGGTACTTCTACACCGCCTTGTCCCGACCTGCAATCAGCG  280
                                                                                                                    lySerHisSerLeuArgTyrPheTyrThrAlaLeuSerArgProAlaIleSerG

AACCGTGGTACATAGCTGTGGGCTACCTGGATGACACTCAGTTCGTGCGCTTCAACAGCTCAGGGGAGACTGCGACATATAAGCTAAGTGCGCCATGGGTGGAGCAAGAGGGGCCCGAGTATTGGGCGAGAGAGACAGAG  420
luProTrpTyrIleAlaValGlyTyrLeuAspAspThrGlnPheValArgPheAsnSerSerGlyGluThrAlaThrTyrLysLeuSerAlaProTrpValGluGlnGluGlyProGluTyrTrpAlaArgGluThrGlu

ATCGTCACAAGCAATGCACAGTTTTTCCGCGAGAATCTGCAGACTATGCTGGACTACTACAACCTGAGTCAAAATGGTGAGGAACCCCCCAAATCTAAGAGTCTGTCCCCACTCCTCGTCCCTCTTATGCAAGCCACCTA  560
IleValThrSerAsnAlaGlnPhePheArgGluAsnLeuGlnThrMetLeuAspTyrTyrAsnLeuSerGlnAsnG
                                                                                                                    EXON 3
ATCATGGGGTGCAAAAGGGAACGGTTTCCCTTTCAGTTTGGAGAATTCTAGGGTGGGCGGCCTGACTAGGAGAGGGGGGCCAGGCTCTCACACCATCCAGGTGATGTATGGCTGTGAGGTGGAGTTCTTCGGGAGCCTCT  700
                                                                                                                    lySerHisThrIleGlnValMetTyrGlyCysGluValGluPhePheGlySerLeuP

TCCGCGCTTATGAGCAGCATGGCTATGATGGCCGCGATTACATTGCCCTCAATGAAGATCTGAAAACGTGGACAGCAGCAGACACGGCAGCAGAGATCACCAGAAGCAAGTGGGAGCAGGCTGGTTATACAGAGCTCCGT  840
heArgAlaTyrGluGlnHisGlyTyrAspGlyArgAspTyrIleAlaLeuAsnGluAspLeuLysThrTrpThrAlaAlaAspThrAlaAlaGluIleThrArgSerLysTrpGluGlnAlaGlyTyrThrGluLeuArg

AGGACCTACTTGGAGGGCCCATGCAAGGATTCCCTGCTCAGATACCTGGAGAACAGAAAAAAGACACAGGAGTGCACAGGTGCGTGGCTGCAGACAACTCCATCTCCCTTTGCCTCTCTACTGGGTTCAGTCCAGAGAGA  980
ArgThrTyrLeuGluGlyProCysLysAspSerLeuLeuArgTyrLeuGluAsnArgLysLysThrGlnGluCysThrA

GCAGCAAACTCACCTAGGTGGGAAAACTCCCATCTCTTTTTCTTTTTTCTTTTTGATTTTTGCTTGTTTGGTTGGTTTTTTTGTTTTTTAATTTTAAAAGGTGACAACAGAAAAATAACGTTAGAAAATTACACAAACCA 1120
ATGGCTATACACAGCTCAGTTGACGTTTGTTAAACAGTTAGAACTGCACTTTGGACCAGTAGAGAACATGAATTTACATATTTCCATTAATATACTCATGCTATCCAACATCGACCATGCAGGCAGGAGAGAGAGACAGA 1260
GACAGAGACTCACAGACACAGAGAGACAGAGGCAGAGGCAGAAGGATGAGAGACAGAAACAGAGAATCAGTGGCAGAATTAGAAGTAGCTATCAATTATTCCCTGCCATTATCCATCGTGTACTTTACCTACATTTAGTC 1400
CTTGCTCATCTCCAAATATTAGCCTTTCCTGATTTCATTATGTCATGTTGCAAGTAACAACAGGTACATTTCTTAACAAGTATACGTTTTTTTGTTTCTTTTGTTTGTTCTTTTGTTTGTTTGTTTGCTAAATTACCTC 1540
TATGAGCAAAAAAGACTGCAGTTTTACTTCCTGTTGTTGTTCTGAGATTGGGTGACTTCGTTTAATATTGTATATTCAAAGTTCATCAATTTTCCTAGATAATTGTGTGATTACATTGTGTAGTGCTCAACAGGATTCCA 1680
CTTTATATATATACCACATTTTGTTAGTCACTTAAGTGAATAATGGGTTGCTTTACTGCAATAGCTAAGATGTCACACTCCATATCAAGGAAGAGAGTAGAGAGTGGACAGTTCTTTCTTTCTTTTTTTTAAAGATTTAT 1820
TTATTTATTTACTTTATTATAACATAAGTACGCTGTAGCG----------800BP----------CTCTTTGCCTCCTGGACACATAGCCTCCTCCTGGCACAAGTTCTGGAATTTCAGAGCATGTATGTAACCT 1928

                                                                                                                : EXON 4
ACTGGGAAATTTGACCCTTGTTCCAGTCATTTCCTTCCCAGTGGCCTCACAGACACTTCTGGAGTCACCAGGAAAGATATTTGATGCCCAAACTTTCCAACTCTTCTCCTCAGATCCTCCAAAAACACACGTGACCCATC 2068
                                                                                                                    spProProLysThrHisValThrHisH

ACCCCAGACCTGAAGGTTATGTCACCCTGAGATGCTGGGCCCTGCGCTTCTACCCTGCTGACATCACCCTGACCTGGCAGTTGAATGGGGAGGAATTGATTCAGGACACGGAGCTTGTGGAGACCAGGCCTGCAGGGGAT 2208
isProArgProGluGlyTyrValThrLeuArgCysTrpAlaLeuArgPheTyrProAlaAspIleThrLeuThrTrpGlnLeuAsnGlyGluGluLeuIleGlnAspThrGluLeuValGluThrArgProAlaGlyAsp

GGAACCTTCCAGAAGTGGGCAGCTGTGGTGGTGCCGCTTGGGAAGGAGCAGAAATACACATGTCATGTGTACCATGAGGGGCTGCCTGAGCCTCTCACCCTGAGATGGGGTAAGGAGGGTGTGGGTGCAGAGCTGGGGTC 2348
GlyThrPheGlnLysTrpAlaAlaValValValProLeuGlyLysGluGlnLysTyrThrCysHisValTyrHisGluGlyLeuProGluProLeuThrLeuArgTrpG

                                                                                                                EXON 5
AGGGAAACCTGGACGCCTTCTGAAGACCCTCAGCTGGTCAGAGTTGGGAATTGAGATCATGTTGCTCATTTTCCCCTCCTGTCCTTACCCTTCCCTTCCCAGAGCTTCCTCAGACCAGTATGCCCAACAGGACCACTGTT 2488
                                                                                                                    luLeuProGlnThrSerMetProAsnArgThrThrVal

CGTGCTCTCCTTGGAGCTATGATCATCTTAGGTTTTATGAGCGGAAGTGTTATGATGTGGATGAGAAAGAACAATGGTATGGAAGAGTCTGTGGCTGGGTGCCTTATGATTGTAAACCAATACACATGCACTCTATGCAA 2628
ArgAlaLeuLeuGlyAlaMetIleIleLeuGlyPheMetSerGlySerValMetMetTrpMetArgLysAsnAsnG

AAGAATTGCCTATCTCTGGGTCTGTTTGCAGACACTCACCTTACAATAAAAGACAGATTCATAATGGTAATGGGGGGTTCTGCACGAGGATTCTAAAATAGCCTGTCAAGTAAGGCAAGCTCATGCTATAAACACTACAG 2768

                                                                                                                EXON 6
ATCTCAGGCTGGCCTTGTTTCATCCCCTGCATATAATGTCCATGGCACAGAACTTTCCTTGGTCTCTCATATCTTTTTTTTTTTAAACAGGTGGAAACGGAGACGATAACACTGCTGCATATCAGAATGAGAGGGAACAC 2908
                                                                                                                    lyGlyAsnGlyAspAspAsnThrAlaAlaTyrGlnAsnGluArgGluHis

TTGTCCCTGACGCCTCGGGCTGAATCTGAGGCACTCGGGGTGGAAGCTGGGATGAAGGATCTTCCTTCTGCCCCACCATTGGTCTCCTGACAATGTCTTGTTACTTCTTCTACTGCAGCGAAGGACAGCTTCTCTCTTGT 3048
LeuSerLeuThrProArgAlaGluSerGluAlaLeuGlyValGluAlaGlyMetLysAspLeuProSerAlaProProLeuValSer .

CTCTGGATACTTGAAAAGGTGACACCTGGGAGCCTGGGATTGGGGCTGAGGCAAACTGGAAAGGACTGGGTCACTGGGCCTTTCCTATTTAAACCTTTGAACGAGAGGAAGGCCTTTGAGATGTTTTCACATTGACTGCC 3188
CTGACTCTTGTTCTTCATCTTGGGACAGCTGCCACATGGGACTGAGTCACTAGTTCTGACTTCTGAACAGTGGAATCACTTTGGGCAGCCGTGCTCGTTTTATGCTTTTGACTATGCATACAATGGGAAAATCTGGAGAG 3328
CACAGCCTGCCCCTGCACACCAAGACTCAGACCCTACATTAGCTGTCATCCCTTGCACAGCCAACCTTCCTGGTCCTCAGGCATTGGGGGAGATCTTCATCCTGTCAGCTCCACGCTGTCCTGGGCGTGTGTCTCCTCAC 3468
ACGCACAGTGTGTCTACTCATTTCCCCACTGAACATGAGAATCAGAATGTGGATGTCAGGCAGTGGTGGACAGGCCATTAATCCTAGCACTTGGGAGGCAGAGGCAGGCAGATTTCTGAGTTCGAGGGCAGCCTGGTTTA 3608
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CTGAGGCCCTGAATTCAATCCCCAGCAACCACATGGTGCTCACAACCATATGTAATAGCATGCAATGCTTCTTCTGGTGTGTTGGAAGTCACCTACAGTGTACTCACCTACATGAAATAAATGAATCTTAAGAAAAGTAT 3888
GAGTGTGAACTCCTTGACCTGAGCAGTTCACTTGTGGGTAAATTAAAGGATTGAGAATATCTGAGTATTGTGGAGGAAATAAATGGCAGCATGGAGGACCTTCCAGAATCTGTGTTTCTTGTGCTGAGTGTGTCAGGTGG 4028
GCAGGAGTCCTCTGGCATCTGCGTGTGAATGGGCTGTGCCAGGTGGAGCTCAGTCCAATGTGAACGTCCCTGCTGGTCAGGTCATTCCTCAGCTATACCTCCTTGGTACTTGTCACTTAATAGAGTCACTGATGACAGAC 4168
TCTGATCATAGGAACACAGCATGGCCTTAGCTTTCTCTTATGACCAGGACTATGAGCCCCTAAGTCTGTACTAGGCCTGTAGTGCAGCTGAGAAACTGACATTACCAGTGAAGAAATTTCGGAAGAGTGGTGGTCAACAC 4308
ATTTAATCCCAACATTCAGGAGACAGACAAGAGAACCACTTGATTTCCAGGCCATCCTGGACACTCAGGGTGACACAGAGAAACCCTGTCTCCAAACTGGGTACCTGGGAGCAGGGTATCCCTGTTGCCAGCCTTGACCG 4448
TGCAGATGATTGGCAGGATGTAGATTTTGGGTTTTGGATTAGAAGAACGTTGCATGCATGAAGCAAGGCTTAAATAGCCAAGCTAGTGGAAGGCGTG 4544
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FIG. 7. DNA sequence of *C25.1*, a *TL* gene of C57BL/6 origin. The deduced amino acid sequence is shown below the DNA sequence. The TGA termination codon is at position 2996 and followed by a 900-bp 3′ untranslated region. The B1 and B2 *Alu*-like repeats are at positions 3523–3701 and 3702–3884. Polyadenylylation signals are at positions 3864 (AATAAA), 3928 (ATTAAA), and 3965 (AATAAA). Position numbers on the right have not been adjusted for DNA segments that have not been sequenced (represented by dashes).

isolated with inserts of 200 bp to 1.8 kb. Sequencing data indicate that at least three *TL* genes are transcribed in ASL1 and two in ERLD (19).

Structure of *TL* Genes. Two pTL1$^+$ clones (C25 and C250) were derived from a cosmid library constructed from ERLD. A major 24S mRNA detected by pTL1 was found in L cells transfected with C25, and TL1 and -2 antigen expression was demonstrated by absorption analysis. C25 contains four class I genes, only one of which is pTL1$^+$. This pTL1$^+$ gene (*C25.1*) was subcloned and transfection and serological analyses showed that *C25.1* codes for TL1 and -2.

Structure and restriction maps of *C25.1* are shown in Fig. 6 and its DNA sequence is shown in Fig. 7. Exon/intron assignments were determined by comparison with TL cDNA clones (19) or, in the case of exon 1, intron 1, and the beginning of exon 2, by comparison with the structure of the *H-2K^b* gene (20). With the exception of intron 3 and exon 6, there is a close similarity between the structure of *C25.1* and *H-2K^b*. Sequence homology between *C25.1* and *H-2K^b* is 64% in exon 1, 69% in intron 1, 73% in exon 2, 60% in intron 2, 76% in exon 3, 91% in exon 4, 77% in intron 4, 63% in exon 5, and 38% in intron 5. Intron 3 of *C25.1* and *H-2K^b* have similar sizes but little sequence homology. With regard to exon 6, *C25.1* has a distinctly different structure from the corresponding region of *H-2K^b* and other class I genes (20, 21). In *C25.1*, exon 6 consists of a single 140-bp coding sequence followed by a 900-bp untranslated region. In *H-2K^b*, this region is divided into exons 6, 7, 8 with a 424-bp 3′ untranslated sequence and two introns (20). Sequences similar to exon 7, exon 8, intron 6, and intron 7 of *H-2K^b* were found in the 3′ untranslated region of *C25.1*, although the homology is rather low. In *C25.1* two tandemly arranged *Alu*-like repetitive sequences, B1 (22) and B2 (23), are also found in the 3′ untranslated region. Two consensus poly(A) signals (AATAAA) and one variant signal (ATTAAA) are found 900–1000 bp downstream from the stop codon of exon 6 of *C25.1*. The R2 *TL* gene of λ17.3/C6.3 has been partially sequenced and shows strong homology with *C25.1*. This indicates that *C25.1* isolated from a TL$^+$ leukemia arising in a TL$^-$ strain has not undergone gross structural rearrangements. The deduced amino acid sequence coded by *C25.1* is also shown in Fig. 7 and it indicates that *C25.1* can code for a polypeptide of M_r 41,000 with two interchain disulfide bridges and up to four carbohydrate chains. This primary structure is similar to the *H-2K^b* product (24).

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