Highly Restricted Expression of the Thymus Leukemia Antigens on Intestinal Epithelial Cells

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Summary

The TL region of the major histocompatibility complex of the mouse contains dozens of tandemly arranged class I genes, including those encoding the thymus leukemia (TL) antigens. TL antigens have been thought to be expressed only on the surface of some T lineage cells, namely immature thymocytes of some mouse strains (TL^+ strains), some leukemia cells, and activated T cells. While the function of TL antigens is unknown, recent studies have implicated the products of at least some TL region class I genes as molecules that present antigens to γ/δ T cells. Since some γ/δ T cells are known to be specifically associated with certain epithelial tissues, we have investigated the expression of some TL region class I genes in a variety of epithelium-containing tissues. Our results show that the TL antigen gene of C57BL/6 mice, $T3^b$, and the TL antigen genes of BALB/c mice, $T3^d$ (previously $T3^c$) and $T18^d$ (previously $T13^c$), are highly expressed in the epithelium of the small intestine. In the case of $T3^b$, we further show, using a *T3* product-specific antibody, that its product is expressed on the surface of the columnar epithelial cells. In addition, we demonstrated that two other TL region class I genes of C57BL/6 origin, $T9^b$ and $T21^b$, are also expressed nearly exclusively in intestinal epithelial cells. These results are consistent with the hypothesis that the products of these TL region class I genes are recognized by γ/δ T cell receptors of intestinal intraepithelial lymphocytes, a subset of γ/δ T cells that is localized in the intestinal epithelium and has a restricted Vγ repertoire. Finally, our study indicates that the relative levels of expression of the two homologous TL antigen genes, $T3^d$ and $T18^d$, differ widely between the thymus and the intestine.

The TL region of the MHC was initially defined as the mouse genetic locus controlling the expression of antigens restricted to the thymus and some leukemias (hence, TL antigens) (1, 2). Subsequent studies showed that TL antigens are expressed on the surface of normal immature thymocytes of some strains (referred to as TL^+), such as BALB/c strain, but not of some other mouse strains, such as C57BL/6 (TL^- strains) (3). Although more recent studies demonstrated the expression of these antigens in activated peripheral T cells (4), their overall tissue distribution seems to be highly restricted. Molecular genetic studies revealed a large number of tandemly arranged class I genes designated *T1-T24* in the TL region (5–7). Transfection experiments indicated that one of these class I genes, *T3*, encodes the TL antigens (8–10) both in C57BL/6 and BALB/c (*T3* alleles of these two mouse strains are referred to as $T3^b$ and $T3^d$, respectively). In addition, BALB/c carries another TL region class I gene referred to as $T18^d$, which is highly homologous to *T3* and also encodes TL antigens (11, 12). No C57BL/6 counterpart of $T18^d$ has been found (9, 12).

While it is well established that products of classical MHC class I genes (H-2K, -D, and -L) present antigen-derived peptides to $CD8^+$ α/β T cells, the function of structurally homologous TL region class I products is poorly understood. However, recent studies from several laboratories suggest that products of at least some TL region class I genes serve as ligands or peptide-presenting molecules for γ/δ T cells (13–17). Since γ/δ T cells associated with different peripheral sites often express a distinct subset of TCRs encoded by a particular combination of γ and δ genes (18–20) or by a subset of γ genes (21–23), one might expect that some TL region class I products would be expressed in these peripheral sites in a tissue-specific and cell type–specific manner (24, 25). With this hypothesis in mind, we reinvestigated the expression of several selected TL region class I genes in a variety of tissues, particularly in those tissues where γ/δ T cells are known to be localized. We report here that some TL region class I genes, including *T3*, are selectively expressed in gut epithelium, a tissue with which a distinct γ/δ T cell subset is known to be associated (21–23).

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Materials and Methods

Mice, Cells, and Antibodies. C57BL/6 and BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Leukemia cells derived from C57BL/6, ERLD, and mAb HD168 were kindly provided by Drs. Elisabeth Stockert, Yuchi Obata, and Lloyd Old (the Samuel Freeman Laboratory, Memorial Sloan-Kettering Cancer Center, New York, NY). HD168 was raised against A strain leukemia, ASL1, and recognizes L cells transfected with native $T3^b$ gene and with chimeric $T3^b$ under the transcriptional control of an H-2K promoter, but does not react with L cells transfected with the thymidine kinase gene alone (8, 26). Furthermore, the $T3^b$ transfectants were recognized by conventional TL typing sera for TL specificity (26). The C57BL/6 trophoblast cell line (27) was obtained from Dr. Keiko Ozato (National Institute of Child Health and Human Development, Bethesda, MD).

RNA Isolation and DNA Constructions. Cellular RNA was isolated from various tissues and cell lines using guanidinium thiocyanate/CsCl method (28). The plasmid used to make the $T3^b$ probe was constructed by inserting the SacI-PstI fragment containing exon 3 of $T3^b$ from cosmid H11 (29) into pBluescript KS^+ (Stratagene Corp., La Jolla, CA). The plasmid containing exon 3 of $T9^b$ was obtained by subcloning the SmaI-SacI fragment from cosmid LSK14 (29) into pBluescript KS^+. Cosmids H11 and LSK14 were kindly provided by Dr. Richard Flavell (Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT).

RNase Protection Assays. Radioactively labeled antisense RNA probes synthesized from plasmids containing exon 3 of $T3^b$ and exon 3 of $T9^b$ were hybridized to the RNAs (10 μg) isolated from various tissues and cells. Yeast tRNA was used as negative control. The hybridization mixes were digested with RNase A and RNase T1 and subsequently fractionated on 5% denaturing polyacrylamide gel according to the standard protocol (30).

Immunohistochemistry. Fresh tissues from 12-wk-old C57BL/6 mice were snap-frozen, and 8-μm sections were fixed with cold acetone, stained with the anti-T3 mAb, HD168, and affinity-purified goat anti–rat antibody linked to colloidal gold particles (AuroProbe LM; Amersham Corp., Arlington Heights, IL). The signal was enhanced with silver reaction following manufacturer's instruction. Dark brown silver grains were generated. The sections were finally counterstained with methyl green.

Polymerase Chain Reaction. cDNA was synthesized from 2 μg of total RNA of thymus or small intestine of C57BL/6 or BALB/c mice, or leukemia line ERLD, using a cDNA cycle kit (Invitrogen Corp., San Diego, CA). One fourth of each cDNA synthesis reaction was used directly for PCR. PCR reaction was performed using Taq polymerase and a thermal cycler (Perkin-Elmer-Cetus, Norwalk, CT). The primers for $T3^b$, $T3^d$, and $T18^d$, 5′ TACACCGCCTTG-TCCCGACCTGC3′ and 5′ AGAGGCTCCCGAAGAACTCCA-CC3′, were derived from the exon 2 and exon 3, respectively. The sequences covered by the primers are identical in all three genes, but $T3^d$ and $T18^d$ differ by 5 bp within the fragments generated by PCR, and $T3^b$ and $T3^d$ are identical within the region (M. Chorney, personal communication). The PCR products were fractionated on a 2% agarose gel, and appropriate fragments were excised and electroeluted in a unidirectional electroelutor (International Biotechnology Inc., New Haven, CT). The fragments were subcloned into the SmaI site of pBluescript and sequenced using Sequenase version 2 kit (U.S. Biochemicals Corp., Cleveland, OH).

Results

$T3^b$ and $T9^b$/$T21^b$ Are Selectively Expressed in Intestinal Epithelium. As shown in Fig. 1, we confirmed the absence of $T3^b$ RNA in C57BL/6 thymus and spleen (8), and its presence in the C57BL/6-derived leukemia line ERLD (8, 9). Most importantly, we found a strong $T3^b$ RNA signal in the small intestine, particularly in its epithelium. Several other organs and tissues where γ/δ T cells are known to be distributed (e.g., lung, uterus, and epidermis) did not give any detectable $T3^b$ RNA signal in this assay (Fig. 1). We also examined the tissue distribution of RNA derived from two additional TL region genes, $T9^b$ and $T21^b$, which are highly homologous in nucleotide sequence (M. Wu and L. Van Kaer, unpublished observation). RNase protection assays showed that these RNAs are present in the small intestine and are enriched in intestinal epithelium (Fig. 2). In addition, very low levels of both mRNAs were observed in the kidney. No $T9^b$ or $T21^b$ RNA was detected in any of the other organs or tissues tested, including the thymus.

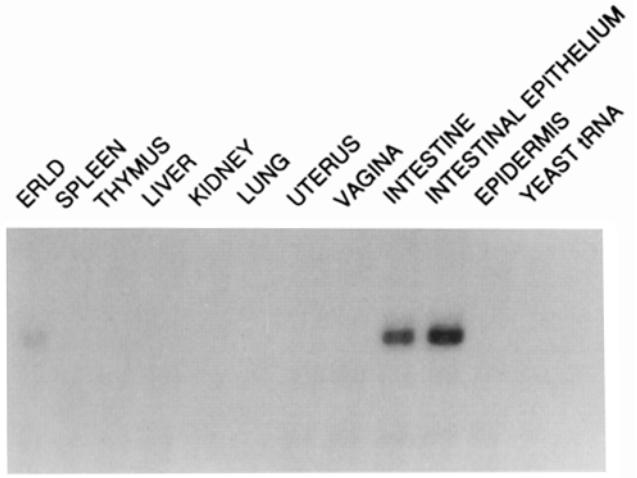

Figure 1. Exclusive expression of $T3^b$ in intestine demonstrated by RNase protection assay. A protected RNA fragment of 88 nucleotides in length was detected in RNA prepared from small intestine, intestinal epithelial cells, and leukemia cells ERLD.

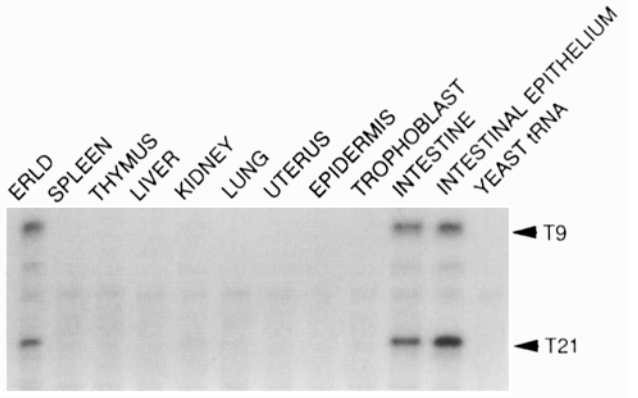

Figure 2. Preferential expression of $T9^b$ and $T21^b$ in the intestine illustrated by RNase protection assay. Protected RNA fragments of 124 and 113 nucleotides, corresponding to $T9^b$ and $T21^b$ message, respectively, were detected in intestine, intestinal epithelial cells, kidney, and ERLD cells.

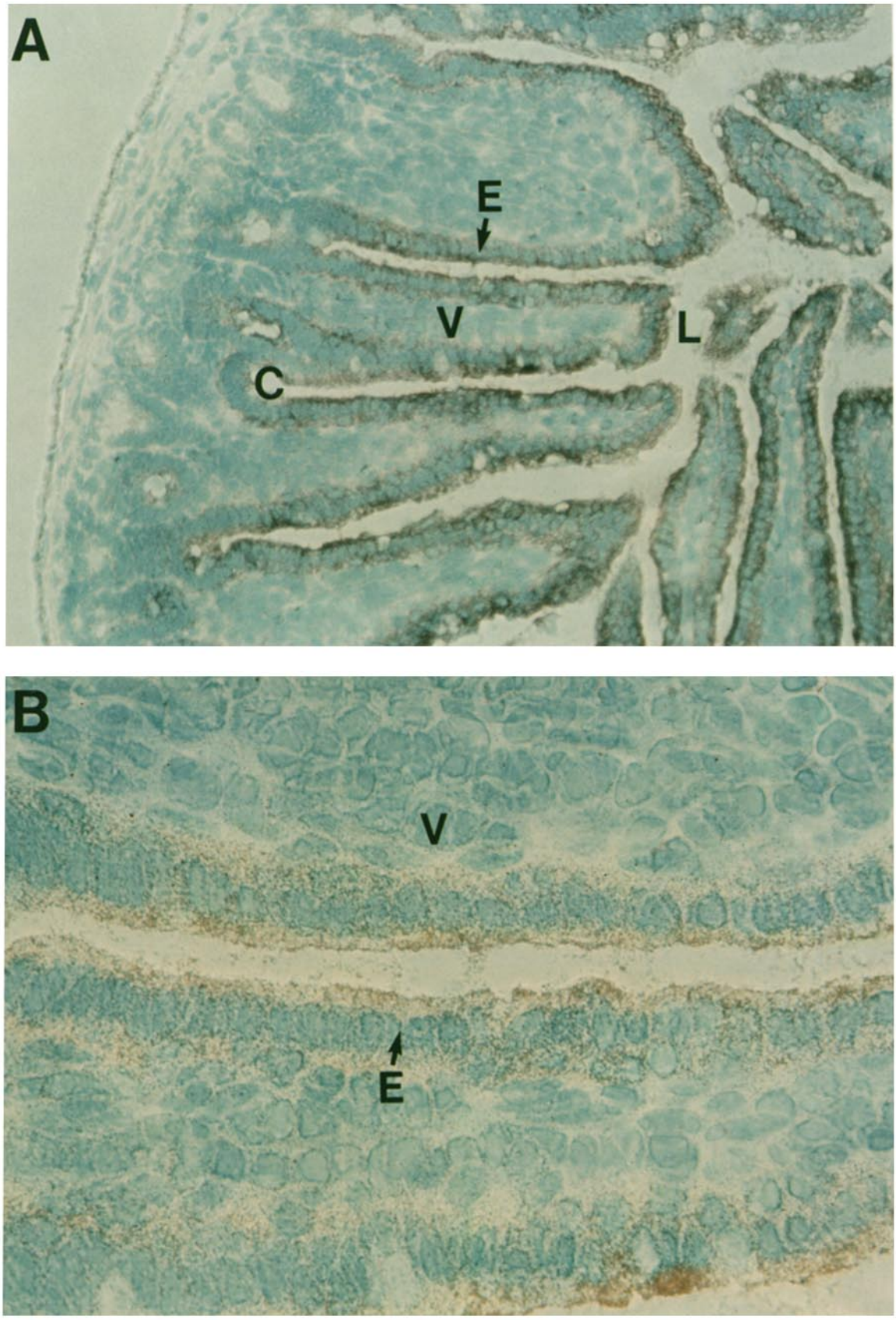

Figure 3. Demonstration of surface expression of $T3^b$ in epithelium of small intestine by immunogold silver staining. (*A*) Transverse section at low magnification; (*B*) transverse section at high magnification. *L*, lumen; *V*, villus; *E*, epithelial cells; and *C*, crypt.

$T3^b$ Molecules Are Expressed on the Surface of Intestinal Epithelial Cells. Immunohistochemical analyses of frozen sections of the small intestine and several other tissues derived from C57BL/6 mice were conducted using a T3-specific mAb, HD168 (8). As shown in Fig. 3, the surface of columnar epithelial cells of the small intestine was stained strongly by HD168. Villus epithelia stained much more intensely than crypt epithelia. Other intestinal tissues appeared to be negative (Fig. 3). Staining was observed on all faces of columnar epithelial cells, on the basolateral membrane, and on the brush border. Staining intensity was highest at the brush border (Fig. 3). Omission of the anti-T3 antibody abolished the immunostaining (data not shown). A similar staining pattern was observed in sections taken from both jejunum (stomach proximal) and ileum (stomach distal) of the small intestine (data not shown). As summarized in Table 1, C57BL/6-derived thymus, spleen, and uterus were all negative for staining by HD168. Sections of large intestine showed some staining, mostly in the intestinal gland (Table 1). No significant staining was observed in the epithelia of the large intestine. A previous study has shown that most of the γ/δ intestinal intraepithelial lymphocytes (i-IELs)[1] are closely associated with villus epithelial cells apparently in contact with the basolateral face (31).

Both $T3^d$ and $T18^d$ Encode TL Antigens in the Intestinal Epithelium, While Most Intrathymic TL Antigens Are Encoded by $T18^d$. To examine whether the observed expression of TL antigens in the intestinal epithelium of C57BL/6 can be extended to BALB/c and to assess the relative contribution of $T3^d$ and $T18^d$ genes in encoding the putative BALB/c TL antigens, we examined cDNAs synthesized from the RNA of small intestine and, for comparison, of thymus of BALB/c mice, by PCR and nucleotide sequencing. The results shown in Table 2 indicate that $T3^d$ and $T18^d$ transcripts are present in approximately equal amounts in the small intestine, suggesting that both TL class I genes encode TL antigens on the intestinal epithelial cells. We confirmed that $T18^d$ is abundantly expressed in thymus. In contrast, $T3^d$ is poorly expressed in BALB/c thymus, similar to $T3^b$ in C57BL/6 thymus. We did detect a very low level of $T3^b$ RNA in the C57BL/6 thymus using the highly sensitive PCR method, which was not detected by the less sensitive RNase protection assay (Fig. 1). This result is consistent with a recent report that the thymus of C57BL/6 mice bears a very low amount of TL antigens (32).

[1] *Abbreviations used in this paper:* IE, intestinal epithelium; i-IEL, intestinal intraepithelial lymphocyte; TL, thymus leukemia.

Table 1. *Tissue Distribution of T3 Molecules*

Organ	Surface staining by anti-T3 mAb	Positive staining cell type
Thymus	–	
Spleen	–	
Uterus	–	
Small intestine	+	Villus (and gland) epithelium
Large intestine	+/–	Gland epithelium

Table 2. *Expression of $T3^d$, $T18^d$, and $T3^b$ in Different Tissues Demonstrated by PCR and DNA Sequencing*

Strain	Tissue	$T3^d$	$T18^d$	$T3^b$
BALB/c	Thymus	1/9	8/9	–
	Intestine	6/10	4/10	–
C57BL/6	Thymus	–	–	3/3
	Intestine	–	–	4/4
	ERLD	–	–	3/3

The number under each gene is expressed as the number of a given cDNA over total number of cDNAs analyzed in a tissue.

Discussion

We have shown that a TL class I gene of C57BL/6 mice, $T3^b$, is expressed on the surface of the epithelial cells of the small intestine (IE cells). This expression is highly tissue- and cell type–specific: several other tissues and cells examined did not show any sign of expression except for a very low level of expression in thymus, which was detected only by highly sensitive PCR analysis. This finding is novel since earlier studies indicated that $T3^b$ expression is restricted to the surface of certain leukemia cells, except for a very low level of expression in the thymus of normal C57BL/6. We have also shown that the highly preferential expression of TL antigens in intestinal epithelium among nonthymus tissues is not a phenomenon restricted to so-called TL^- mouse strains such as C57BL/6. Thus, BALB/c, a TL^+ strain, seems to also express $T3^d$, the BALB/c counterpart of $T3^b$, in intestinal epithelium. In addition, we have shown that two additional, and highly homologous TL region class I genes, $T9^b$ and $T21^b$, are transcribed nearly exclusively in the small intestine.

The function of the products of these IE-specific TL region class I genes is unknown. It is possible that they are involved in the control of normal differentiation of these highly proliferative cells. An alternative and more attractive possibility is suggested by recent studies on the specificity of γ/δ T cells (14, 16, 17) and by the finding that a particular subset (21–23) of γ/δ T cells (i-IELs) is localized in the intestinal epithelium apparently in contact with the epithelial cells (31). Thus, it has recently been shown that the product of TL region class I gene *T22* is recognized by a γ/δ TCR expressed by a hybridoma derived from a C57BL/6 thymocyte (14). Another recent study indicates that a molecule bearing the Qa-1 antigen, which is probably encoded by the product of another TL region class I gene *T23* (33), presents a Glu-Tyr (GT) copolymer to a T cell hybridoma derived from splenic γ/δ T cells (17). In addition, a product encoded by an unknown TL region gene has been reported to be recognized by an alloreactive γ/δ T cell clone derived from splenic T cells (16). These and other recent observations led to the hypothesis that TL region class I gene products have evolved to present certain endogenous and common microbial antigens to γ/δ T cells (24, 34).

The γ/δ T cells used for these previous studies were derived either from adult thymus or spleen, which contain widely circulating γ/δ T cells with relatively diverse TCRs encoded by multiple Vγ and Vδ gene segments (35). The class I products recognized by these γ/δ T cells, namely T22 and T23, are expressed in a variety of tissues and cells (14, 36). In contrast, γ/δ T cells associated with epithelia seem to be localized in the respective peripheral sites. These latter types of γ/δ T cells are known to express TCRs that are encoded by a particular combination of Vγ and Vδ gene segments (18–20) or by a certain Vγ gene segment (21–23). For example, γ/δ T cells associated with gut epithelium (i-IELs) selectively use the Vγ7 gene segment (21–23). Thus, if TL region class I gene products have indeed evolved as the antigen-presenting molecules for γ/δ T cells, the localization of γ/δ T cell subsets with restricted and distinct TCR repertoire in specific epithelia suggests the possibility of an equally specific and restricted expression of some TL region class I gene products on the surface of these epithelial cells. The present results are compatible with this notion for *T3* and possibly $T9^b$ and $T21^b$, with respect to i-IEL, and suggest that these TL region class I gene products may be recognized by the γ/δ TCR of i-IEL.

After this work was completed, we learned that Hershberg et al. (37) have independently shown that TL antigens are expressed on the surface of intestinal epithelial cells of BALB/c mice. On the basis of PCR and Southern blot analyses, these authors concluded that these TL antigens are encoded by the TL class I gene $T18^d$. However, their methods cannot distinguish $T18^d$ from $T3^d$ expression. Our results, shown in Table 2, indicate that $T3^d$ and $T18^d$ are equally expressed in the intestinal epithelial cells of BALB/c mice, while the TL antigens expressed on the surface of BALB/c thymocytes are primarily encoded by $T18^d$ (Table 2), in agreement with a previously presented hypothesis (10).

It thus appears that the *T3* gene is regulated similarly between TL^- and TL^+ mouse strains: it is poorly expressed in the thymus, its primary site of expression is the surface of intestinal epithelial cells, and its expression is activated in radiation-induced leukemia cells (8, 9). On the other hand, the TL^+ strain BALB/c, but not the TL^- strain C57BL/6, carries an additional TL antigen–encoding gene, $T18^d$, which is expressed both in thymocytes and in intestinal epithelial cells. Although $T3^d$ and $T18^d$ are highly homologous, their gene products differ by 14 amino acids within the three extracellular domains (10), and the $T3^d$ protein has 24 additional amino acids at its COOH terminus (10). These structural differences and the distinct expression patterns suggest different roles.

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