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

By Min Wu, Luc Van Kaer, Shigeyoshi Itohara, and Susumu Tonegawa

From the Howard Hughes Medical Institute at Center for Cancer Research, and the Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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, T3b, and the TL antigen genes of BALB/c mice, T3 (previously T3) and T18 (previously T13), are highly expressed in the epithelium of the small intestine. In the case of T3b, 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, T3 and T21, 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 and T18, 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 T3b and T3d, respectively). In addition, BALB/c carries another TL region class I gene referred to as T18, which is highly homologous to T3 and also encodes TL antigens (11, 12). No C57BL/6 counterpart of T18 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+ αβ 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 (21-22), 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 reinvigated 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).
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 T3b gene and with chimeric T3b 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 T3b 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 guanidine thiocyanate/CsCl method (28). The plasmid used to make the T3b probe was constructed by inserting the SacI-PstI fragment containing exon 3 of T3b from cosmid H11 (29) into pBluescript KS* (Stratagene Corp., La Jolla, CA). The plasmid containing exon 3 of T9b was obtained by subdoning the Smal-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 T3b and exon 3 of T9b 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.

Results

T3b and T9b/T21b Are Selectively Expressed in Intestinal Epithelium. As shown in Fig. 1, we confirmed the absence of T3b 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 T3b 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 T3b RNA signal in this assay (Fig. 1). We also examined the tissue distribution of RNA derived from two additional TL region genes, T9b and T21b, 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 T9b or T21b RNA was detected in any of the other organs or tissues tested, including the thymus.

Figure 1. Exclusive expression of T3b 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 3. Demonstration of surface expression of TPβ 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 epithelium stained much more intensely than crypt epithelium. 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 epithelium of the large intestine. A previous study has shown that most of the \(\gamma/\delta\) intestinal intraepithelial lymphocytes (i-IELs)\(^3\) 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 nontumour 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 T22\(^b\), 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 \(\gamma/\delta\) T cells (14, 16, 17) and by the finding that a particular subset (21–23) of \(\gamma/\delta\) 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 \(\gamma/\delta\) 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 \(\gamma/\delta\) T cells (17). In addition, a product encoded by an unknown TL region gene has been reported to be recognized by an alloreactive \(\gamma/\delta\) 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 \(\gamma/\delta\) 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 T3δ and T21δ, 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 T18δ. However, their methods cannot distinguish T18δ from T3δ expression. Our results, shown in Table 2, indicate that T3δ and T18δ 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 T18δ (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, T18δ, which is expressed both in thymocytes and in intestinal epithelial cells. Although T3δ and T18δ are highly homologous, their gene products differ by 14 amino acids within the three extracellular domains (10), and the T3δ protein has 24 additional amino acids at its COOH terminus (10). These structural differences and the distinct expression patterns suggest different roles.

We thank Drs. Elisabeth Stockert, Yuchi Obata, and Lloyd Old for ERLD cells and mAb HD168; Dr. Richard Flavell for cosmids H11 and LSK14; Dr. Keiko Ozato for the B6 trophoblast cell line; Drs. Hiroshi Mashimo, Michael J. Chorney, and Stanley Nathenson for providing the T3d nucleotide sequence information before publication; Dr. Andrew G. Farr for advice on immunostaining; Drs. Donal Murphy and Dietmar Kappes for critical reading of the manuscript; and Elly Basel for secretarial assistance.

M. Wu and S. Itohara are supported by the Howard Hughes Medical Institute, and L. Van Kaer is supported by the Cancer Research Institute. This work was supported by grants from the National Institutes of Health, Howard Hughes Medical Institute, and Yakult Honsha Co., Ltd.

Address correspondence to Susumu Tonegawa, Howard Hughes Medical Institute, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, 77 Massachusetts Avenue, Cambridge, MA 02139.

Received for publication 4 March 1991.

References
1. Old, L.J., E.A. Boyse, and E. Stockert. 1963. Antigenic properties of experimental leukemias. I. Serological studies in vitro with spontaneous and radiation-induced leukemias. J. Natl. Cancer Inst. 31:977.
2. Boyse, E.A., L.J. Old, and S. Luell. 1964. Genetic determination of the TL (thymus-leukemia) antigen in the mouse. Nature (Lond.) 201:779.
3. Old, L.J., and E. Stockert. 1977. Immunogenetics of cell surface antigens of mouse leukemia. Annu. Rev. Genet. 17:127.
4. Cook, R.G., and N.F. Landolfi. 1983. Expression of the thymus leukemia antigen by activated peripheral T lymphocytes. J. Exp. Med. 158:1012.
5. Flaherty, L., E. Elliott, J.A. Tine, A.C. Walsh, and J.B. Waters. 1990. Immunogenetics of the Q and TL regions of the mouse. Crit. Rev. Immunol. 10:131.
6. Stroynowski, I. 1990. Molecules related to class-I major histocompatibility complex antigens. Annu. Rev. Immunol. 8:501.
7. Klein, J., C. Benoist, C.S. David, P. Demant, K.F. Lindahl, L. Flaherty, R.A. Flavell, U. Hämmerling, L.E. Hood, S.W. Hunt III, P.P. Jones, P. Kourilsky, H.O. McDevitt, D. Meruelo, D.B. Murphy, S.G. Nathenson, D.H. Sachs, M. Steinmetz, S. Tonegawa, E.K. Wakeland, and E.H. Weiss. 1990. Revised nomenclature of mouse H-2 genes. Immunogenetics. 32:147.
8. Obata, Y., Y.-T. Chen, E. Stockert, and L.J. Old. 1985. Structural analysis of TL genes of the mouse. Proc. Natl. Acad. Sci. 1990.
Selective Expression of Thymus Leukemia Genes in Intestinal Epithelium

USA. 82:5475.

9. Pontarotti, P.A., H. Mashimo, R.A. Zeff, D.A. Fisher, L. Hood, A. Mellor, R.A. Flavell, and S.G. Nathenson. 1986. Conservation and diversity in the class I genes of the major histocompatibility complex: sequence analysis of a Tla gene and comparison with a Tla gene. *Proc. Natl. Acad. Sci. USA.* 83:1782.

10. Chorner, M.J., H. Mashimo, Y. Buskskin, and S.G. Nathenson. 1989. Characterization of the thymus leukemia (TL) product encoded by the BALB/c T3 gene by DNA-mediated gene transfer comparison to the T3 product and BALB/c leukemia TL. *J. Immunol.* 143:3762.

11. Goodenow, R.S., M. McMillan, M. Nicolson, B.T. Sher, K. Eakle, N. Davidson, and L. Hood. 1982. Identification of the class I genes of the mouse major histocompatibility complex by DNA-mediated gene transfer. *Nature (Lond.)* 300:231.

12. Fisher, D.A., S.W. Hunt III, and L. Hood. 1985. Structure of a gene encoding a murine thymus leukemia antigen, and organization of TL genes in the BALB/c mouse. *J. Exp. Med.* 162:528.

13. Bonneville, M., K. Ito, E.G. Krecko, S. Itohara, D. Kappes, I. Ishida, O. Kanagawa, C.A. Janeway, Jr., D.B. Murphy, and S. Tonegawa. 1989. Recognition of a self major histocompatibility complex TL region product by γδ T-cell receptors. *Proc. Natl. Acad. Sci. USA.* 86:5928.

14. Ito, K., L. Van Karer, M. Bonneville, S. Hsu, D.B. Murphy, and S. Tonegawa. 1990. Recognition of the product of a novel MHC TL region gene (27th) by a mouse γδ T cell receptor. *Cell.* 62:549.

15. Bluestone, J.A., R.Q. Cron, M. Cotterman, B.A. Houlden, and L.A. Matis. 1988. Structure and specificity of T cell receptor γδ on major histocompatibility complex antigen-specific CD3+, CD4−, CD8+ T lymphocytes. *J. Exp. Med.* 168:1899.

16. Houlden, B.A., L.A. Matis, R.Q. Cron, S.M. Widacki, G.D. Brown, C. Pampeno, D. Meruelo, and J.A. Bluestone. 1989. A TCR γδ cell recognizing a novel TL-encoded gene product. *Cold Spring Harbor Quant. Symp. Biol.* 49:45.

17. Vidović, D., M. Roglič, M. McKune, S. Guerder, C. MacKay, and Z. Dembič. 1989. Qa-1 restricted recognition of foreign antigen by a γδ T-cell hybridoma. *Nature (Lond.)* 340:646.

18. Koning, F., G. Stingl, W.M. Yokoyama, H. Yamada, W.L. Maloy, E. Tschachler, E.M. Shevach, and J.E. Coligan. 1987. Identification of a T3-associated gamma delta T cell receptor on Thy-1+ dendritic epidermal cell lines. *Science (Wash. DC).* 236:834.

19. Kuziel, W.A., A. Takashima, M. Bonyhadi, P.R. Bergstresser, J.P. Allison, R.E. Tigelaar, and P.W. Tucker. 1987. Regulation of T-cell receptor γ-chain RNA expression in murine Thy-1+ dendritic epidermal cells. *Nature (Lond.)* 328:263.

20. Itohara, S., A. Farr, J.L. Lafaille, M. Bonneville, Y. Takagaki, W. Haas, and S. Tonegawa. 1990. Homing of a γδ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature (Lond.)* 343:734.

21. Bonneville, M., C.A. Janeway, Jr., K. Ito, W. Haser, I. Ishida, N. Nakashishi, and S. Tonegawa. 1988. Intestinal intraepithelial lymphocytes are a distinct set of γδ T cells. *Nature (Lond.)* 336:479.

22. Takagaki, Y., A. DeCloux, M. Bonneville, and S. Tonegawa. 1989. Diversity of γδ T-cell receptors on murine intestinal intraepithelial lymphocytes. *Nature (Lond.)* 339:712.

23. Asarnow, D.M., T. Goodman, L. LeFrancois, and J.P. Allison. 1989. Distinct antigen receptor repertoires of two classes of murine epithelium-associated T cells. *Nature (Lond.)* 341:60.

24. Tonegawa, S., A. Berns, M. Bonneville, A. Farr, I. Ishida, K. Ito, S. Itohara, C.A. Janeway, Jr., O. Kanagawa, M. Katsuki, R. Kubo, J. Lafaille, P. Mombaerts, D. Murphy, N. Nakashishi, Y. Takagaki, L. Van Karer, and S. Verbeek. 1989. Diversity, development, ligands, and probable functions of γδ T cells. *Cold Spring Harbor Quant. Symp. Biol.* 49:31.

25. Van Karer, L., M. Wu, Y. Ichikawa, K. Ito, M. Bonneville, S. Ostrand-Rosenberg, D.B. Murphy, and S. Tonegawa. 1991. Recognition of MHC TL gene products by γδ T cells. *Immunol. Rev.* 120:51.

26. Obata, Y., E. Stockert, Y.T. Chen, T. Takahashi, and L.J. Old. 1988. Influence of 5' flanking sequences on TL and H-2 expression in transfected L cells. *Proc. Natl. Acad. Sci. USA.* 85:3541.

27. Tanaka, K., K. Ozato, G. Jay, J.R. Parnes, L. Ramanathan, J.G. Seidman, K.S.S. Chang, and E. Appella. 1983. Control of H-2 antigen and β2-microglobulin gene expression in mouse trophoblast cell clones. *Proc. Natl. Acad. Sci. USA.* 80:5597.

28. Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonuclease from sources enriched in ribonuclease. *Biochemistry.* 18:5294.

29. Weiss, E.H., L. Golden, K. Fahmer, A.L. Mellor, J.J. Devlin, H. Bullman, H. Tidens, H. Bud, and R.A. Flavell. 1984. Organization and evolution of the class I gene family in the major histocompatibility complex of the C57BL/10 mouse. *Nature (Lond.)* 310:650.

30. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith, and K. Struhl. 1987. Current Protocols in Molecular Biology. Section 4–7. Greene/John Wiley & Sons, New York.

31. Bonneville, M., S. Itohara, E.G. Krecko, P. Mombaerts, I. Ishida, M. Kutsuki, A. Berns, A.G. Farr, C.A. Janeway, Jr., and S. Tonegawa. 1990. Transgenic mice demonstrate that epithelial homing of γδ T cells is determined by cell lineages independent of T-cell receptor specificity. *J. Exp. Med.* 171:1015.

32. Michaelson, J., E.A. Boyse, L. Cicco, L. Flaherty, E. Fleissner, E. Garnick, U. Hämmerling, M. Lawrence, P. Mauch, and F.W. Shen. 1986. Biochemical genetics of TL antigens. *Immunogenetics.* 24:103.

33. Wolf, P., and R.G. Cook. 1990. The TL region gene 37 encodes a Qa-1 antigen. *J. Exp. Med.* 172:1795.

34. Janeway, C.A., Jr. 1989. Approaching the asymptote? Evolution and revolution in immunobiology. *Cold Spring Harbor Quant. Symp. Biol.* 49:1.

35. Cron, R.Q., F. Koning, W.L. Maloy, D. Pardoll, J.E. Coligan, and J.A. Bluestone. 1988. Peripheral murine CD3+, CD4−, CD8− T lymphocytes express novel T cell receptor γδ structures. *J. Immunol.* 141:1074.

36. Trany, C., S.R. Nash, B. David-Wätine, M. Cochet, S.W. Hunt III, L.E. Hood, and P. Kourilsky. 1987. A low polymorphic mouse H-2 class I gene from the Tla complex is expressed in a broad variety of cell types. *J. Exp. Med.* 166:341.

37. Hershberg, R., P. Eghtesady, B. Sydora, K. Bronson, H. Cheroutre, R. Modlin, and M. Kronenberg. 1990. Expression of the thymus leukemia antigen in mouse intestinal epithelium. *Proc. Natl. Acad. Sci. USA.* 87:9727.