Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 7995–7999, October 1989
Immunology

Expression of TL, H-2, and chimeric H-2/TL genes in transgenic mice: Abnormal thymic differentiation and T-cell lymphomas in a TL transgenic strain

(thymus leukemia antigens/major histocompatibility complex class I antigens/thymus-specific expression/L3T4$^-$ Lyt-2$^-$ thymocytes/T-cell leukemogenesis)

NORIYUKI HAMASIMA*, TOSHITADA TAKAHASHI*, OSAMU TAGUCHI*, YASUAKI NISHIZUKA*, ELISABETH STOCKERT†, LLOYD J. OLD†, AND YUICHI OBATA*‡

*Aichi Cancer Center Research Institute, Chikusa-ku, Nagoya 464, Japan; and †Memorial Sloan–Kettering Cancer Center, New York, NY 10021

Contributed by Lloyd J. Old, May 25, 1989

ABSTRACT **To investigate the genetic regulation of TL expression, 12 transgenic mouse strains on a C3H (TL-nonexpressing) background have been derived: two Tg.Tla^a-3 strains with *Tla^a-3* isolated from A-strain TL$^+$ thymocytes, four Tg.T3^b strains with *T3^b* from a TL$^+$ leukemia arising in a C57BL/6 (TL$^-$) mouse, three Tg.Con.3 strains with an *H-2K^b/T3^b* chimeric gene (construct 3, 5′ flanking region and exon 1 of *H-2K^b* and exons 2–6 of *T3^b*), one Tg.Con.4 strain with a *T3^b/H-2K^b* chimeric gene (construct 4, 5′ flanking region and exon 1 of *T3^b* and exons 2–8 of *H-2K^b*), and two Tg.H-2K^b strains with *H-2K^b*. Expression of the transgenes was determined by the presence of TL or H-2K^b products or transcripts. Both Tg.Tla^a-3 strains expressed high levels of TL antigen in thymus, indicating that (*i*) the 9.6-kilobase *Tla^a-3* DNA fragment contains sufficient information for correct tissue-specific expression in thymocytes and (*ii*) TL$^-$ thymocytes of C3H provide conditions for the transcriptional activation of *Tla^a-3*. In contrast, neither the four Tg.T3^b strains nor the Tg.Con.4 strain expressed transgenes, indicating that (*i*) *T3^b* lacks elements necessary for TL expression in normal thymocytes and (*ii*) the corresponding endogenous TL genes of C3H mice also lack these elements. The pattern of TL expression in two of the three Tg.Con.3 strains was similar to that of H-2K^b expression, indicating that transcription of this *H-2K^b/T3^b* chimeric gene was driven by the regulatory sequences of *H-2K^b*. The thymuses of mice derived from the Tg.Tla^a-3-1 strain were smaller than C3H thymuses, and the surface phenotype of Tg.Tla^a-3-1 thymocytes resembled thymocyte precursors (TL$^+$ L3T4$^-$ Lyt-2$^-$ Thy-1$^+$ H-2$^+$). These mice developed a high incidence of lymphomas with the same thymocyte precursor phenotype. The study of TL transgenic strains should prove useful in defining the role of TL in normal and abnormal T-cell differentiation.**

TL (thymus leukemia) antigens are coded for by genes in the major histocompatibility complex (MHC) of the mouse (1). Although TL is similar in overall structure to other class I MHC antigens (H-2, Qa), TL expression is regulated in a highly distinctive fashion. In contrast to the broad distribution of H-2 antigens and the intermediate distribution of Qa antigens, TL expression is restricted to T cells during development in the thymus and is lost when T cells migrate to the periphery. Some mouse strains do not express TL antigens on thymocytes (TL$^-$ strains), but leukemias occurring in these mice can have a TL$^+$ phenotype, indicating activation of normally silent TL genes.

To examine the structural basis for the differential expression of TL, we and others have cloned and sequenced TL genes from various mouse strains (2–5). Further, we have constructed chimeric genes consisting of TL and H-2 sequences and analyzed the expression of these genes in transfected L cells (6). These studies showed that expression-competent TL genes exist in TL$^-$ as well as TL$^+$ strains and that differential expression of TL and H-2 in L cells is due to regulatory sequences in the 5′ flanking region of the genes. These studies also showed that the TL genes are regulated differently in L cells than in thymocytes or leukemia cells and that the initiation and processing of TL transcripts in L cells are aberrant. In the present study, we have extended our analysis of TL regulation to transgenic mice, and 12 transgenic strains carrying TL, H-2, and TL/H-2 chimeric genes have been derived and characterized.

MATERIALS AND METHODS

TL, H-2, and Chimeric Genes. A clone containing *Tla^a-3* was isolated from a library of A-strain thymocytes (Y.O., Y.-T. Chen, E.S., and L.J.O., unpublished observation). Detailed description of other clones containing *T3^b*, *H-2K^b*, and chimeric genes was presented previously (6) and is summarized in Table 1. Fragments used for microinjection are described in the legend of Fig. 1.

Production and Maintenance of Transgenic Mouse Strains. Two picoliters of DNA suspension (10 μg/ml) was microinjected into male pronuclei of fertilized eggs obtained from C3H/He mice (Charles River, Japan Inc., Hino), following the method of Gordon *et al.* (7). Southern blot analysis of DNA isolated from tails was used to detect the presence of transgenes. Positive mice were mated with C3H/He, and positive mice of the second or third generation were mated with each other to produce homozygous mice.

Serological Analysis. Cell surface antigens were analyzed by fluorescence-activated cell sorter (FACS) (Becton Dickinson, FACStar instrument) with rat monoclonal antibodies to TL (HD168 and HD177, ref. 2), L3T4 (53.6.72, ref. 8), Lyt-2 (GK1.5, ref. 9), and H-2 (HD464; E.S., Y.O., and L.J.O., unpublished observation) and with mouse monoclonal antibodies to H-2K^b (HB11, ref. 6) and Thy-1.2 (purchased from Becton Dickinson). In cytotoxicity assays, (BALB/c × C3H/An)F$_1$ anti-ASL1 leukemia serum preabsorbed *in vivo* in (C57BL/6 × A-*Tla^b*)F$_1$ mice carrying ERLD leukemia was used to detect TL3, and (A × B6-*Tla^a*)F$_1$ anti-ERLD was used to detect TL4 (1, 10).

DNA and RNA Blot Analyses. Probes for Southern (11) and Northern (12) blot analyses were prepared either by nick-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: FACS, fluorescence-activated cell sorter; MHC, major histocompatibility complex.

‡To whom reprint requests should be addressed.

Table 1. Derivation and characteristics of 12 TL and H-2 transgenic strains

Gene (origin; designation)	Antigen encoded	Derived strain	No. of copies*	Expression of transgene (positive tissues)†
Tla^a-3 (A strain; p50H)	TL	Tg.T1a^a-3-1	7	+ (thymus, lymph node, and spleen)
		Tg.T1a^a-3-2	13	+ (thymus, lymph node, and spleen)
T3^b (B6 TL$^+$ leukemia ERLD; p20)	TL	Tg.T3^b-1	26	−
		Tg.T3^b-2	10	−
		Tg.T3^b-3‡	24	−
		Tg.T3^b-4	22	−
H-2K^b (B6; pc1.4.1)	H-2K^b	Tg.H-2K^b-1	7	+ (lymph node, lung, and others)
		Tg.H-2K^b-2	2	+ (lymph node, lung, and others)
H-2K^b/T3^b (chimeric; construct 3)	TL	Tg.Con.3-1	23	+ (lymph node, lung, and others)
		Tg.Con.3-2	19	+ (lymph node, lung, and others)
		Tg.Con.3-3	12	−
T3^b/H-2K^b (chimeric; construct 4)	H-2K^b	Tg.Con.4-1	7	−

*Determined by Southern blot analysis.
†Determined by antigen expression and presence of transcript.
‡The founder mouse of Tg.T3^b-3 had the transgene in two independent chromosomal sites with 24 and 5 copies, respectively. Offspring of this mouse having a single integration site with 24 copies were selected to derive Tg.T3^b-3.

translation (13) or by 5′ end labeling (14). Conditions for hybridization and washing have been described (2, 4).

RESULTS AND DISCUSSION

Derivation of Transgenic Mouse Strains. Pronuclei of fertilized eggs of C3H/He (TL$^-$ and H-2K^k) were microinjected with five different genes or gene constructs (Fig. 1). Twelve transgenic mouse strains were thus derived: two strains with *Tla^a-3*, four with *T3^b*, two with *H-2K^b*, three with the *H-2K^b/T3^b* chimeric gene, and one with the *T3^b/H-2K^b* chimeric gene (Table 1). Southern blot analysis of founder mice of the transgenic strains showed that all contained multiple copies of transgenes tandemly arrayed in a head-to-tail configuration. All founder mice, with one exception, had transgenes integrated into a single chromosomal site. The one exception had transgenes integrated into two independent chromosomal sites, and only one strain was derived from this founder. Transgenes were inherited in a Mendelian fashion in the offspring of the transgenic mice derived in this study.

Expression of Transgenes. Tissues of transgenic mice were tested for the presence of TL or H-2K^b antigens by FACS analysis (Figs. 2 and 3) and for the presence of TL and H-2 RNA transcripts by Northern blot analysis (Figs. 4 and 5). All transgenic mice used for analysis of expression were 2 months old and hemizygous for the transgenes. A summary of the results is shown in Table 1. In general, the presence of antigen and transcript correlated well, although the level of antigen expression was not always proportional to the amount of transcript. In Tg.Tla^a-3-1 and Tg.Tla^a-3-2 mice, high levels of TL antigen and TL transcripts were found in thymus, with low but significant amounts detected in lymph nodes and spleen; no TL antigen or TL transcripts were detected in bone marrow or other tissues. Cytotoxicity assays confirmed these results with lymphoid cells and showed that thymocytes of both strains expressed TL3 but not TL4 specificity. Expression of TL3 verifies coding of TL by the *Tla^a-3* transgene rather than by activation of endogenous TL genes of C3H/He mice; the TL3 specificity has never been detected outside of strains normally expressing TL3 on thymocytes (1, 10). The size of the major transcripts in Tg.Tla^a-3-1 and -3-2 mice was 2.4 kb, which corresponds to the less prominent TL transcripts found in thymocytes of A-strain (*Tla^a*) and C57BL/6 (B6) congenic mice (B6-*Tla^a*). These results indicate that the 9.6-kb fragment of *Tla^a-3* used for derivation of the transgenic strains contains sufficient

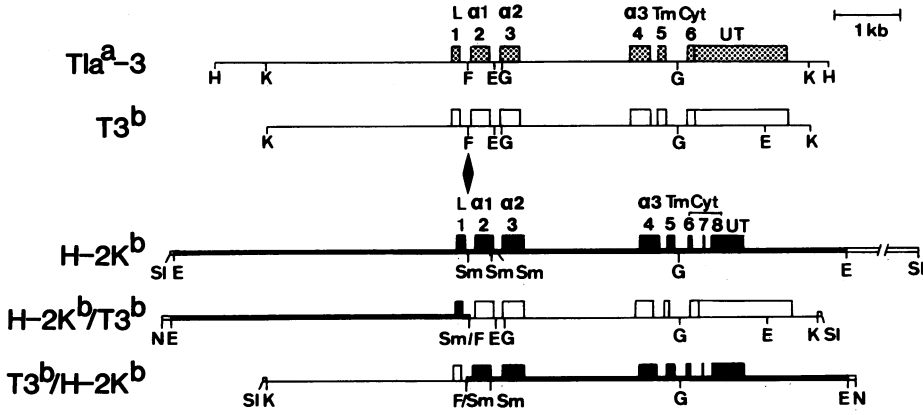

FIG. 1. Characteristics of TL, H-2, H-2/TL, and TL/H-2 transgenes. *Tla^a-3* and its 3.5-kilobase (kb) 5′ and 0.8-kb 3′ flanking regions are contained in a clone of a 9.6-kb *Hin*dIII–*Hin*dIII fragment. Clones containing *T3^b* and *H-2K^b*, and construction of the two chimeric genes, have been described (6). For microinjection, the following fragments were used: a 9.6-kb *Hin*dIII–*Hin*dIII fragment containing *Tla^a-3*; an 8.5-kb *Kpn* I–*Kpn* I fragment containing *T3^b*; a 13.2-kb *Sal* I–*Sal* I fragment containing 10.5 kb of *H-2K^b* and 2.7 kb of vector sequence; a 10.1-kb *Nar* I–*Sal* I fragment containing 9.9 kb of *H-2K^b/T3^b* chimeric sequence (construct 3) and 161 and 21 base pairs (bp) of vector sequence at the 5′ and 3′ end, respectively; and a 9.2-kb *Sal* I–*Nar* I fragment containing 9 kb of *T3^b/H-2K^b* chimeric sequence (construct 4) and 21 and 161 bp of vector sequence at the 5′ and 3′ end, respectively. Exons represented by numbered stippled (*Tla^a-3*), open (*T3^b*), or filled (*H-2K^b*) boxes encode leader (L), extracellular (α1, α2, α3), transmembrane (Tm), and cytoplasmic (Cyt) domains of the proteins or the 3′ untranslated (UT) region. Open bars represent pUC19 vector sequences. Restriction enzyme sites: E, *Eco*RI; F, *Fsp* I; G, *Bgl* II; H, *Hin*dIII; K, *Kpn* I; N, *Nar* I; Sl, *Sal* I; Sm, *Sma* I.

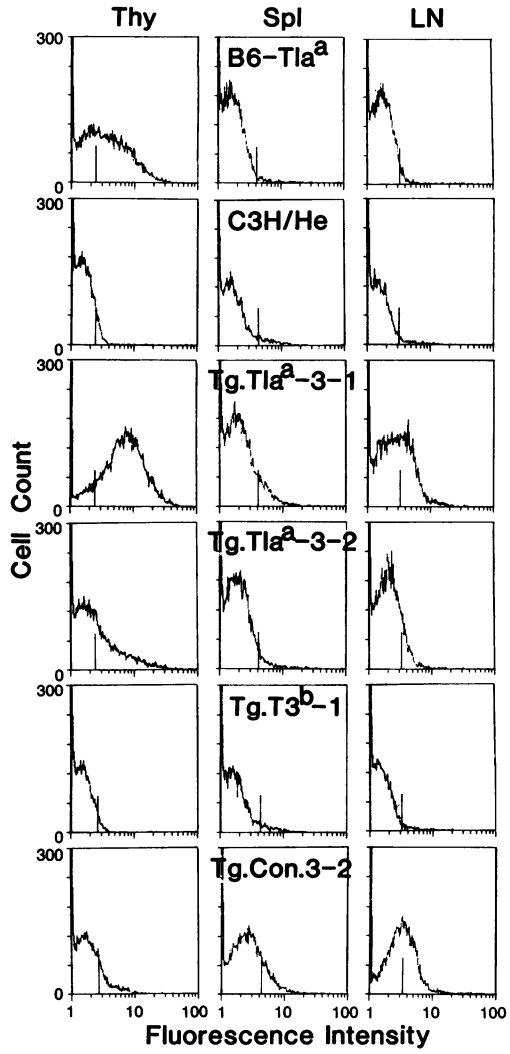

FIG. 2. Expression of TL antigen in transgenic mouse strains. Cells from thymus (Thy), spleen (Spl), and lymph nodes (LN) were tested for the expression of TL by FACS analysis with rat monoclonal TL antibodies (HD168 and HD177). Essentially identical results were obtained with HD168 and HD177; only the results with HD168 are shown. The four Tg.T3^b strains did not express TL, and Tg.Con.3-1 and Tg.Con.3-2 strains expressed TL in an identical pattern; for illustration, the results with Tg.T3^b-1 and Tg.Con.3-2 are shown. B6-*Tla^a* (TL$^+$ strain) and C3H/He (TL$^-$ strain) were used as positive and negative controls (see Table 1).

information for the preferential tissue-specific expression of TL in thymus and that the thymic environment of C3H/He (a strain that does not express TL) provides an inducing environment for the expression of the TL transgene. The expression of TL in lymph nodes and spleen of Tg.Tla^a-3 strains contrasts with the absence of TL expression in extrathymic lymphoid tissues in TL$^+$ inbred strains. Two possibilities could account for this aberrant expression: (*i*) high copy number in Tg.Tla^a-3 strains allows the transgene to escape a suppressing mechanism or (*ii*) the 9.6-kb fragment lacks sequences responsible for suppression of TL expression in peripheral T cells. Derivation of transgenic mice with single or low copy number or with fragments containing larger flanking sequences may clarify these possibilities.

Unlike the *Tla^a-3* transgenic strains, none of the four Tg.T3^b strains expressed TL antigen or TL transcripts in any tissue. Similarly, the Tg.Con.4-1 strain (formed with the chimeric gene containing 5′ flanking sequences of *T3^b* and *H-2K^b* coding sequences) failed to express H-2K^b antigen or transcripts in any tissue, in contrast to abundant H-2K^b

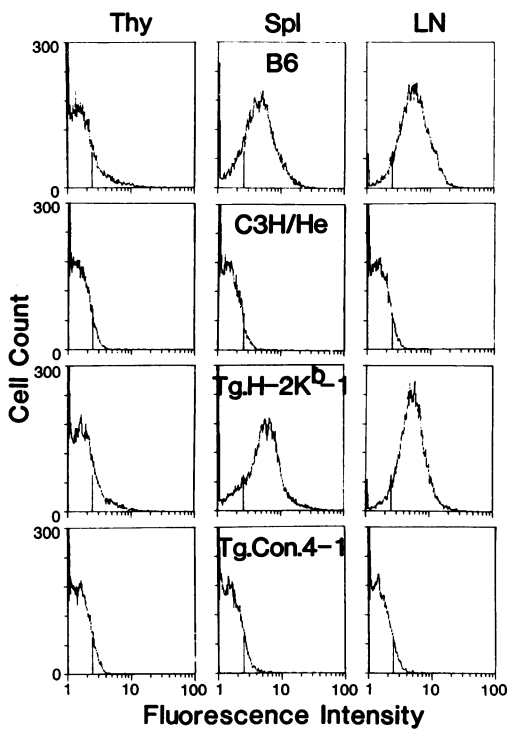

FIG. 3. Expression of H-2K^b antigen in transgenic mouse strains. Cells from thymus (Thy), spleen (Spl), and lymph nodes (LN) were tested for the expression of H-2K^b antigen by FACS analysis with mouse monoclonal H-2K^b antibody. Two Tg.H-2K^b strains expressed H-2K^b antigen in an identical pattern; only the results of Tg.H-2K^b-1 are shown. B6 (*H-2K^b*) and C3H/He (*H-2K^k*) were used as positive and negative controls.

expression in Tg.H-2K^b strains. These results indicate that *T3^b*, like the corresponding endogenous TL genes of C3H/He mice, lacks elements necessary for expression in normal thymocytes or any other tissues. The same fragment containing *T3^b* was expressed in transfected L cells, although initiation and processing of the transcripts were abnormal (6). Thus, expression of the TL transgene is controlled more strictly *in vivo* than in cultured cells. In a comparison of >500 bases of 5′ flanking sequences of *Tla^a*-3 and *T3^b*, only a few base changes have been found.

Of the three Tg.Con.3 strains, Tg.Con.3-1 and Tg.Con.3-2 expressed high levels of TL antigen in lymph nodes and spleen and low levels in thymus. RNA transcripts were detected in almost all tissues and were particularly abundant in lymph nodes, spleen, and lung. This pattern of expression is identical to H-2K^b expression in the two Tg.H-2K^b strains and in B6 (*H-2K^b*). It appears therefore that expression of this *H-2K^b/T3^b* chimeric gene was driven by an *H-2K^b* regulatory unit. The major TL transcripts found in the Tg.Con.3-1 and -2 mice were 2.1 kb, 500 bases longer than the 1.6-kb H-2K^b transcript. As the 3′ untranslated region of *T3^b* is 500 bases longer than that of *H-2K^b*, it seems likely that the chimeric gene is transcribed from the initiation site of *H-2K^b* through the 3′ untranslated region of *T3^b*. TL4 specificity (but not TL3) was expressed by lymph node lymphocytes of Tg.Con.3-1 and Tg.Con.3-2 mice. This antigenic specificity has never been found on normal TL$^+$ thymocytes but is expressed by TL$^+$ leukemias occurring in TL$^-$ mice (1, 11). Expression of TL4 in Tg.Con.3 strains was in accordance with L-cell transfection experiments in that *T3^b* and the *H-2K^b/T3^b* chimeric gene, used to generate Tg.T3^b and Tg.Con.3 strains, code for TL4 (6).

Abnormal T-Cell Differentiation and Leukemia Development in Tg.Tla^a-3-1 Mice. Tg.Tla^a-3-1 mice were observed to

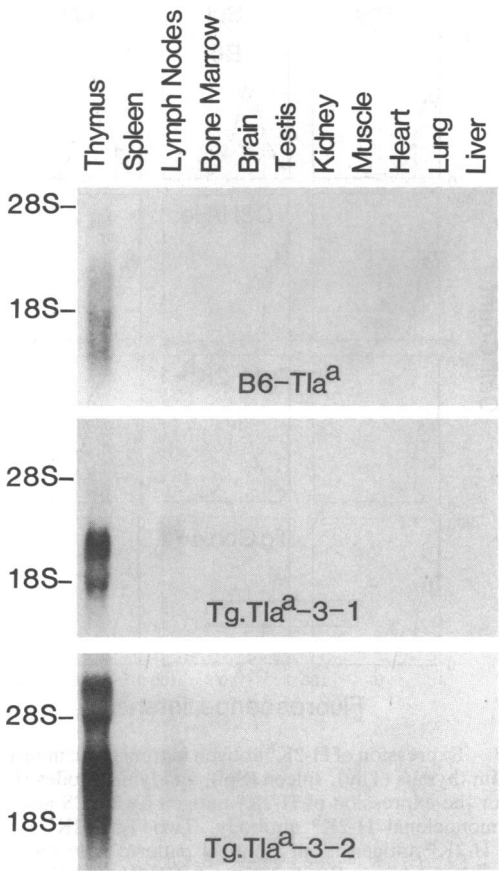

FIG. 4. TL transcripts in tissues of Tg.Tla^a-3 strains. Ten micrograms of total RNA was loaded per lane and analyzed with a TL-specific probe, pTL1 (2). Tissues of B6-*Tla^a* (a TL$^+$ strain) were included for comparison. At least three TL genes, *Tla^a-1*, *Tla^a-2*, and *Tla^a-3*, are expressed in B6-*Tla^a* thymocytes, with major transcripts of 1.3 kb and 1.8 kb and minor transcripts of 2.4 kb (4). In Tg.Tla^a-3 strains, the 2.4-kb transcripts were clearly detected in thymus and also in small amounts in lymph nodes. The 2.4-kb transcripts were detected in spleen, but only after a long exposure time (data not shown). Ribosomal 28S and 18S RNAs were used as markers, corresponding to 4.7 kb and 1.9 kb.

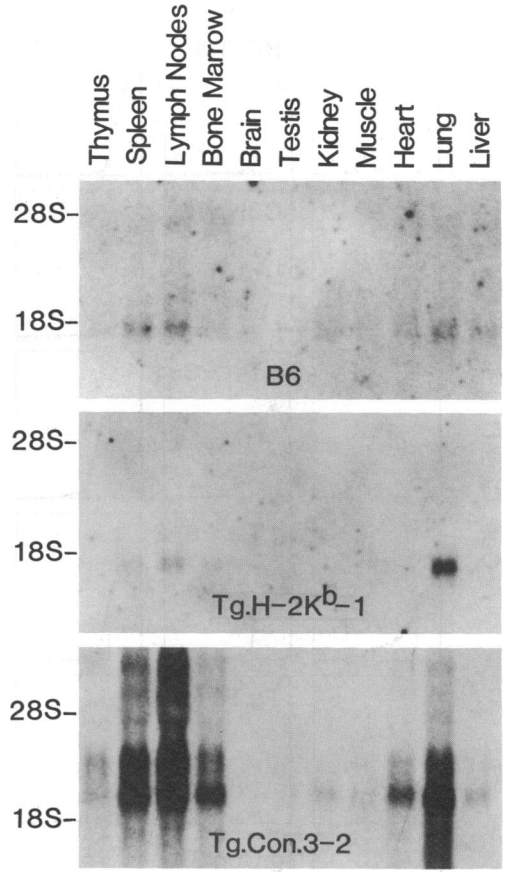

FIG. 5. TL and H-2K^b RNA transcripts in tissues of Tg.H-2K^b and Tg.Con.3 strains. Ten micrograms of total RNA was loaded per lane. Blots of B6 and Tg.H-2K^b-1 RNA were analyzed with an H-2K^b-specific 23-mer oligonucleotide (15). Blots of Tg.Con.3-2 RNA were analyzed with pTL1, a TL-specific probe that hybridizes with exon 5 and exon 6 of *T3^b*. H-2K^b transcripts of control B6 and Tg.H-2K^b-1 were 1.6 kb, and the TL transcripts of Tg.Con.3-2 were 2.1 kb. Tissue distribution of transcripts of transgenes in Tg.H-2K^b and Tg.Con.3 was similar to that of H-2K^b transcripts of B6 mice.

have smaller thymuses than mice of similar age belonging to other strains (e.g., Tg.Tla^a-3-2, C3H/He, or B6-*Tla^a*). As shown in Fig. 6, the thymus of Tg.Tla^a-3-1 mice consists of large blastlike cells with high mitotic activity, and the usual cortical/medullary demarcation characteristic of normal thymus was lacking. The surface phenotype of Tg.Tla^a-3-1 thymocytes was TL$^+$ L3T4$^-$ Lyt-2$^-$ Thy-1$^+$ H-2$^+$. In B6-*Tla^a* and Tg.Tla^a-3-2 mice, >80% of thymocytes were L3T4$^+$ Lyt-2$^+$ (Fig. 7). In normal thymic differentiation, L3T4$^-$ Lyt-2$^-$ thymocytes are thought to be the least differentiated cells of the thymocyte population (representing <5%), which then differentiate to L3T4$^+$ Lyt-2$^+$ cells (80–85%) and then to L3T4$^+$ Lyt-2$^-$ or L3T4$^-$ Lyt-2$^+$ cells (10–15%) (see ref. 16 for a review). Rearrangement and expression of T-cell anti-

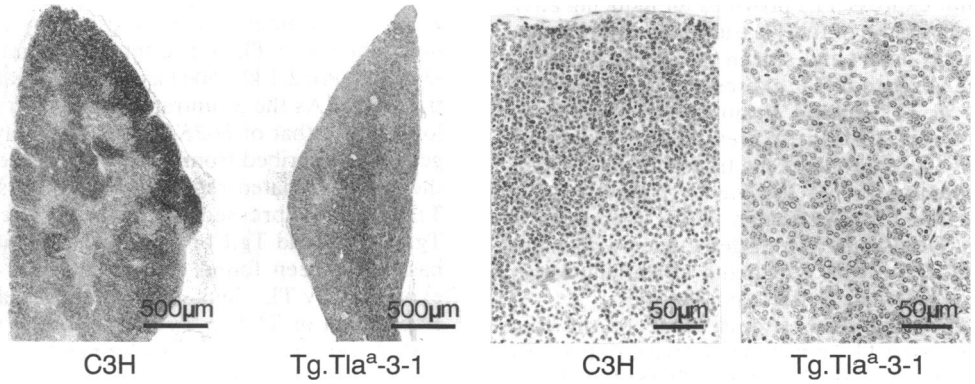

FIG. 6. Histology of thymus of 2-month-old Tg.Tla^a-3-1 and C3H (control) mice. The clear demarcation between cortex and medulla in C3H is absent in Tg.Tla^a-3-1. The thymocyte population in Tg.Tla^a-3-1 mice consists of large blastlike cells in contrast to the small size of C3H thymocytes.

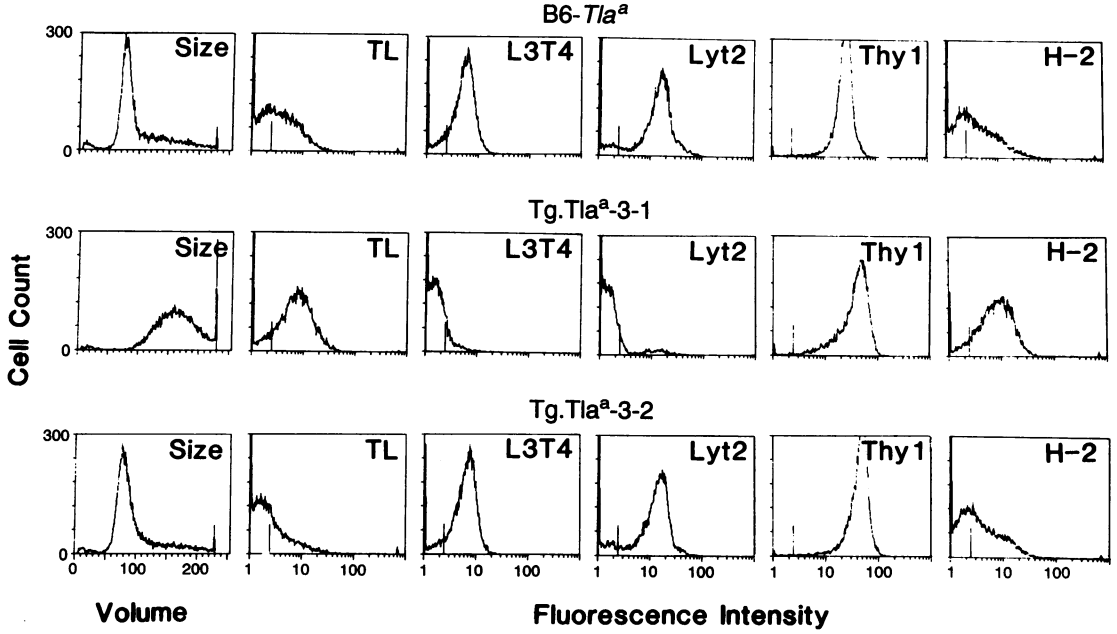

FIG. 7. Size and surface phenotype of thymocytes of Tg.Tla^a-3-1. Antigen phenotype was determined by FACS analysis using corresponding monoclonal antibodies (see *Materials and Methods*). The volume of Tg.Tla^a-3-1 thymocytes was more than twice that of B6-*Tla^a* or Tg.Tla^a-3-2 thymocytes. The majority of Tg.Tla^a-3-1 thymocytes were L3T4$^-$ Lyt-2$^-$ Thy-1$^+$, while control B6-*Tla^a* and Tg.Tla^a-3-2 thymocytes were L3T4$^+$ Lyt-2$^+$ Thy-1$^+$. Expression of TL and H-2 antigens was much higher in Tg.Tla^a-3-1 thymocytes than in B6-*Tla^a* or Tg.Tla^a-3-2 thymocytes.

gen receptor α- and β-chain genes take place during the transition from L3T4$^-$ Lyt-2$^-$ to L3T4$^+$ Lyt-2$^+$ cells (17). Thus, the striking increase of L3T4$^-$ Lyt-2$^-$ thymocytes in Tg.Tla^a-3-1 mice indicates arrest of normal thymic differentiation at a critical early stage. A high incidence of spontaneous lymphomas involving lymph nodes and spleen has been observed in Tg.Tla^a-3-1 mice; leukemias have been found in 19 of 29 mice at 8–15 months. No leukemias have occurred in the Tg.Tla^a-3-2, Tg.T3^b, or Tg.Con.3 transgenic mouse strains, and C3H/He is known to be a strain with a low incidence of lymphoma [≈5% at 24 months (18)]. The leukemias of Tg.Tla^a-3-1 mice are readily transplantable in C3H/He mice. All leukemias tested so far have a surface phenotype comparable to Tg.Tla^a-3-1 thymocytes: TL$^+$ L3T4$^-$ Lyt-2$^-$ Thy-1$^+$ H-2$^+$.

Several explanations can be put forward for the maturational arrest of T cells in Tg.Tla^a-3-1 mice. Insertional mutagenesis fortuitously involving a locus controlling T-cell development is one possibility that cannot be ruled out. If this was the case, insertional activation rather than inactivation of the locus was the result, because the abnormal thymic characteristics occurred in both hemizygous and homozygous Tg.Tla^a-3-1 mice and were inherited as a dominant trait. Inappropriate expression of TL3 in a strain not normally expressing the antigen is another possibility, but this is unlikely because Tg.Tla^a-3-2 and Tg.Con.3 mice also express TL3 and yet show no thymic abnormalities. Nor can the persistence of the TL$^+$ phenotype in post-thymus lymphocytes (in contrast to the usual loss of TL expression in T cells leaving the thymus) be involved in the maturational arrest of Tg.Tla^a-3-1 thymocytes, since it is also seen in Tg.Con.3 and Tg.Tla^a-3-2 mice. A critical difference between Tg.Tla^a-3-1 and the other TL transgenic strains, including Tg.Tla^a-3-2, may be the much higher levels of TL expressed by thymocytes and peripheral T cells of the Tg.Tla^a-3-1 mice. Abnormally amplified levels of TL may suppress subsequent steps in T-cell maturation; this idea can be tested by constructing additional transgenic strains having comparably high levels of TL. Whatever the explanation for the thymic abnormalities observed in Tg.Tla^a-3-1 strains, these mice provide a unique opportunity to examine the L3T4$^-$ Lyt-2$^-$ thymocyte population in detail and to probe the relation between maturational arrest and T-cell leukemogenesis.

We thank Dr. Ken-ichi Yamamura for teaching us microinjection technique, Dr. Yasuo Morishima for assistance in FACS analyses, and Dr. Nobutaka Shinohara for L3T4 and Lyt-2 monoclonal antibodies. We thank Yasue Matsudaira and Hitomi Hasegawa for technical assistance. This research was supported in part by a Grant-in-Aid for Cancer Research and a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science, and Culture, Japan; the Samuel Freeman Charitable Trust, Inc.; and Grant CA08748 from the National Cancer Institute.

1. Chen, Y.-T., Obata, Y., Stockert, E., Takahashi, T. & Old, L. J. (1987) *Immunol. Res.* **6**, 30–45.
2. Obata, Y., Chen, Y.-T., Stockert, E. & Old, L. J. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5475–5479.
3. Fisher, D. A., Hunt III, S. W. & Hood, L. (1985) *J. Exp. Med.* **162**, 528–545.
4. Chen, Y.-T., Obata, Y., Stockert, E. & Old, L. J. (1985) *J. Exp. Med.* **162**, 1134–1148.
5. Pontarotti, P. A., Mashimo, H., Zeff, R. A., Fisher, D. A., Hood, L., Mellor, A., Flavell, R. A. & Nathenson, S. G. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 1782–1786.
6. Obata, Y., Stockert, E., Chen, Y.-T., Takahashi, T. & Old, L. J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 3541–3545.
7. Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 7380–7384.
8. Dialynas, D. P., Quan, Z. S., Wall, K. A., Pierres, A., Quintans, J., Loken, M. R., Pierres, M. & Fitch, F. W. (1983) *J. Immunol.* **131**, 2445–2451.
9. Ledbetter, J. A. & Herzenberg, L. A. (1979) *Immunol. Rev.* **47**, 63–90.
10. Old, L. J. & Stockert, E. (1977) *Annu. Rev. Genet.* **11**, 127–160.
11. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
12. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
13. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113**, 237–251.
14. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
15. Miyada, C. G. & Wallace, R. B. (1986) *Mol. Cell. Biol.* **6**, 315–317.
16. Scollay, R., Wilson, A., D'Amico, A., Kelly, K., Egerton, M., Pearse, M., Wu, L. & Shortman, K. (1988) *Immunol. Rev.* **104**, 81–120.
17. Von Boehmer, H., Karjalainen, K., Pelkonen, J., Borgulya, P. & Rammensee, H.-G. (1988) *Immunol. Rev.* **101**, 21–37.
18. Smith, G. S. & Walfford, R. L. (1978) in *Genetic Effects on Aging*, ed. Bergsma, D. & Harrison, D. E. (Liss, New York), pp. 281–312.