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# T-cell receptor $\gamma\delta$ and $\gamma$ transgenic mice suggest a role of a $\gamma$ gene silencer in the generation of $\alpha\beta$ T cells

**(T-cell lineages/allelic exclusion)**

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**ABSTRACT** **A T lymphocyte expresses on its surface one of two types of antigen receptor, T-cell receptor $\alpha\beta$ or T-cell receptor $\gamma\delta$, encoded by a pair of somatically rearranged $\alpha$ and $\beta$ or $\gamma$ and $\delta$ genes. It has been suggested that $\alpha\beta$ T cells are generated only from precursor T cells that failed to rearrange $\gamma$ and $\delta$ genes in a functional form. However, we found that transgenic mice constructed with functionally rearranged $\gamma$ and $\delta$ genes produce a normal number of $\alpha\beta$ T cells. The transgene $\gamma$ present in these $\alpha\beta$ T cells is repressed apparently through an associated cis DNA element (silencer). We propose that some T-cell precursors are committed to generate $\alpha\beta$ T cells independent of the rearrangement status of their $\gamma$ gene and that this commitment involves activation of a factor(s) that interacts with the $\gamma$ gene-associated silencer.**

The search for the genes encoding the antigen receptor of T cells, T-cell receptor (TCR) $\alpha\beta$, led to the identification of a third set of TCR genes, $\gamma$ (1–3), which in time led to the discovery of a second TCR, TCR $\gamma\delta$ (4–6). One interesting issue raised by the discovery of $\gamma\delta$ T cells is their developmental relationship with $\alpha\beta$ T cells. During thymic ontogeny, rearrangement and cell-surface expression of $\gamma$ and $\delta$ genes precede those of $\alpha$ and $\beta$ genes (7–9). In peripheral $\alpha\beta$ T cells, some $\gamma$ genes are rearranged, often but not always, in a nonproductive (i.e., out-of-frame) form (2, 10–12). In contrast, in $\gamma\delta$ thymocytes or peripheral $\gamma\delta$ T cells, $\beta$ genes are almost always in an incompletely rearranged D–J form (D, diversity; J, joining), while $\alpha$ genes are never rearranged (13–15). In addition, cells bearing both TCR $\alpha\beta$ and TCR $\gamma\delta$ or "hybrid" TCRs such as $\beta\delta$ or $\alpha\gamma$ heterodimers do not exist or are very rare (8, 9, 16). On the basis of these observations, Pardoll *et al.* (8) and Allison and Lanier (17) proposed that if the $\gamma$ and $\delta$ gene rearrangements are both productive, the cells proceed to surface expression of TCR $\gamma\delta$, which, in analogy with the immunoglobulin system (18), inhibits further rearrangement of any other TCR gene. $\alpha\beta$ T cells are generated only from those cells that failed to productively rearrange both $\gamma$ and $\delta$ genes.

We tested this model by analyzing transgenic (Tg) mice constructed with a pair of productively rearranged $\gamma$ and $\delta$ genes cloned from a $\gamma\delta$ T-cell hybridoma. Since all T-cell precursors in these mice should have productively rearranged Tg $\gamma$ and $\delta$ genes, the model would predict that generation of $\alpha\beta$ T cells would be blocked. However, we found that a normal number of $\alpha\beta$ T cells is produced in these Tg mice. The $\gamma$ and $\delta$ Tg are transcriptionally silent in $\alpha\beta$ T cells and the repression of $\gamma$ gene expression seems to be mediated by a cis-acting DNA element (a silencer) present in the flanking regions of the $\gamma$ gene. We suggest that some precursor T cells are programmed to repress the expression of $\gamma$ (and also perhaps $\delta$) genes, which may be either productively or nonproductively rearranged, by a mechanism mediated by the silencer element(s) and that it is from these precursor T cells that $\alpha\beta$ T cells are generated.

## MATERIALS AND METHODS

**Monoclonal Antibodies (mAbs).** Anti-TCR $\gamma\delta$ (3A10) and anti-clonotypic KN6 (5C10) mAbs have been described (9). Anti-TCR $\alpha\beta$ (H57-597) was a gift from R. Kubo (National Jewish Center for Immunology and Respiratory Medicine, Denver) (19).

**DNA Probes and Primers.** The $V_{\gamma4}$, $J_{\delta1}$, and $V_{\delta5}$ (V, variable) probes have been described (15). The $C_\gamma$ and $C_\alpha$ (C, constant) probes have also been described (1). The Thy-I and actin probes were prepared from clone pMT8 (20) and an actin cDNA clone (21), respectively.

**Construction of Tg Mice.** For the production of $\gamma\delta$ double Tg mice, the inserts of the pδ-23/15 and pγ-4 cosmids cloned in the pWE15 vector (22) were dissected by *Sma* I and *Not* I, respectively, and coinjected (4 μg/ml each) into fertilized mouse eggs from superovulated (CBA/BrA × C57BL/LiA)$F_1$ females that had been mated with $F_1$ males (23). The Tg mice were identified by the tail DNA-blotting method using $^{32}$P-labeled $V_{\gamma4}$ and $V_{\delta5}$ probes. The $\gamma$ single Tg mice were similarly prepared by using the *Eco*RI/*Sal* I insert of the phage λ clone pγ-L.

**Southern and Northern Blot Analyses and Quantitation of Band Intensity.** DNA and RNA were purified by the standard procedures and subjected to analysis according to Chomczynski and Qasba (24) using probes labeled by a random priming method (25). The autoradiographic bands were detected and intensities were quantitated by using a Fuji Bioimage analyzer BA100 (26). This machine detects radioactivity recorded on photoexcitable phosphor upon stimulation by a laser beam and is at least 100 times more sensitive than conventional autoradiography with x-ray film for the detection of $^{32}$P. The machine also detects a wide range of radioactivity (50–$10^4$ dpm) in a linear fashion, enabling highly accurate quantitation of band intensity.

**Polymerase Chain Reaction (PCR).** Ten micrograms of total RNA from each T-cell hybridoma was incubated with 0.5 μM synthetic 3′ primer (GTCCATAATGTGAAATACAAT), 0.5 mM dNTPs, 50 mM Tris·HCl (pH 8.3), 10 mM $MgCl_2$, 8 mM dithiothreitol, 2 units of RNasin (Amersham), and 10 units of reverse transcriptase (Amersham) in total vol of 20 μl. After 45 min at 42°C, the reaction mixture was diluted to 100 μl with 50 mM KCl, 10 mM Tris·HCl (pH 8.4), 2.5 mM $MgCl_2$, together with 1 mM synthetic 5′ primer (CTCTCTAACTCA-

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Abbreviations: TCR, T-cell receptor; Tg, transgenic or transgene(s); mAb, monoclonal antibody; D, diversity; J, joining; V, variable; C, constant; PCR, polymerase chain reaction.

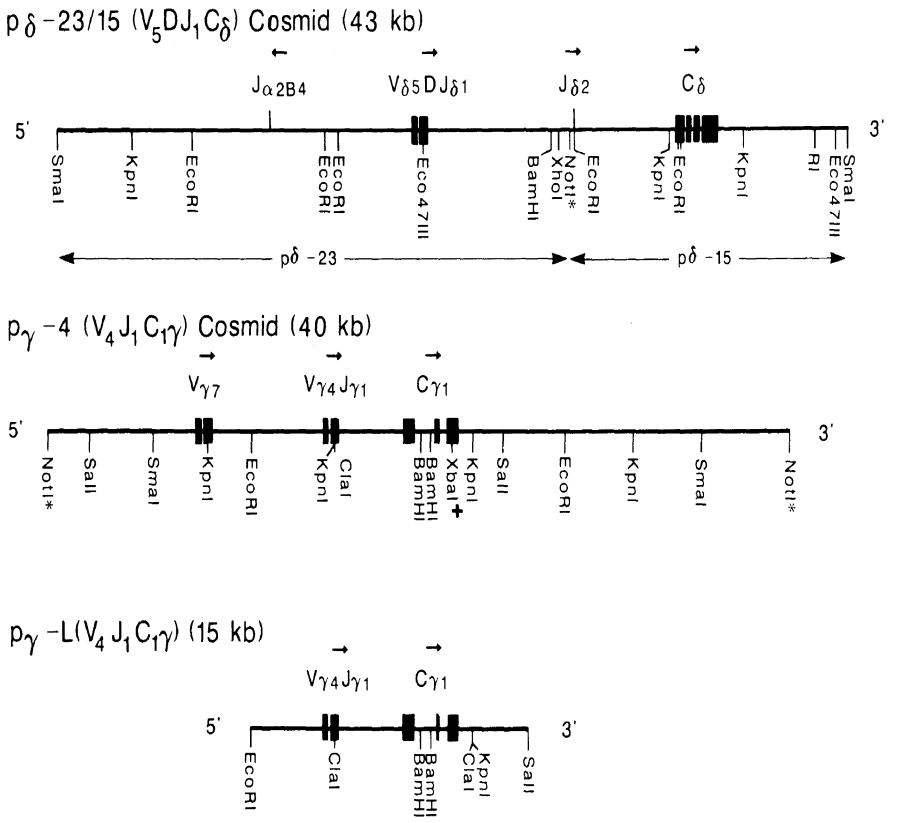


FIG. 1. Restriction maps of γ and δ genomic DNA clones. The cosmids, pδ-23/15 and pγ-4, containing the productively rearranged $V_5DJ_1C$ δ and $V_4J_1C_1$ γ genes, respectively, were cloned from genomic DNA from a C57BL/6 γδ T-cell hybridoma KN6. pδ-23/15 is a composite clone constructed from two primary clones pδ-23 and pδ-15. The *Not* I* sites were created during the cloning procedures. The phage λ clone pγ-L contains another $V_4J_1C_1$ γ gene expressed on the surface of another T-cell hybridoma 3D10 (C3H). The *Xba* $I^+$ site present in the 3′ untranslated region of the $C_{\gamma 1}$ gene segment was destroyed in clone pγ-L by a filling-in reaction with Klenow DNA polymerase. The *Xba* I and *Eco*RI maps of clone pγ-4 are incomplete. Solid boxes designate exons or gene segments and arrows above them indicate the direction of transcription.

CATCACCTC), 250 μM dNTPs, and 0.5 unit of *Thermus aquaticus* DNA polymerase (*Taq* polymerase) (Perkin–Elmer/Cetus), PCR cycles were run according to ref. 27 with 1 min at 92°C, 2 min at 50°C, and 3 min at 72°C.

**Flow Cytometry and Fluorescence-Activated Cell Sorting.** Flow cytometry was carried out as described (9) using a FACScan (Becton Dickinson). For the purification of αβ T cells, splenic T cells from γδ-1355 mice were enriched by a nylon wool column and stained with biotin-coupled anti-αβ mAb and phycoerythrin-streptavidin and sorted. The purification of γδ T cells from γδ-1355 mice was carried out as described (28).

**Generation of T-Cell Hybridomas.** For the generation αβ and γδ hybridomas, total thymocytes or splenic T cells and $CD4^-$ $CD8^-$ (double negative) thymocytes were fused with an αβ TCR-negative thymoma BW5147 (29) as described (14).

## RESULTS

**Construction of γδ Double Tg Mice.** Three γδ double Tg mice (γδ-1355, γδ-1314, and γδ-1313) were obtained by coinjection of two cosmid clones, Pγ-4 and Pδ-23/15 (Fig. 1), which carry the γ and δ genes, respectively, encoding the TCR of a γδ T-cell hybridoma KN6 (14). The three Tg lines carry a single to a few copies of the injected γ and δ genes (Table 1). Their splenocytes and thymocytes (Table 1) but not livers (data not shown) contain elevated levels of γ and δ RNA of appropriate sizes, suggesting that the Tg are expressed in the lymphoid organs of these mice. The presence of Tg-encoded RNA in the thymus was confirmed by a separate experiment in which synthetic oligonucleotide probes specific to the V–J or V–D–J junctional sequences of the Tg were used (data not shown).

**An Increased but Still Minor Proportion of Thymocytes and Splenic T Cells Expresses the Tg on the Cell Surface.** The total number of thymocytes and splenocytes is equivalent between the Tg mice and their non-Tg littermates (Table 2). Using appropriate mAbs in flow cytometry, we quantitated the γδ TCR- and αβ TCR-expressing cells among the various cell populations. Less than 0.2% of thymocytes and <1% of splenocytes from normal mice bear γδ TCRs, and cells bearing KN6 γδ TCRs are too low to be detected in either cell population (Table 2; ref. 9). By contrast, in the γδ Tg mice, 2–3% of thymocytes and 3–4% of splenocytes bear γδ TCRs, of which a large proportion (80–100%) is KN6 clonotype positive (Table 2). These results strongly suggest that the Tg-encoded TCRs dominate the γδ T-cell population. However, γδ T cells still compose a relatively small proportion (<4%) of thymocytes and splenocytes in the Tg mice.

Indeed, analysis with the αβ TCR mAb indicated that neither the absolute number nor the proportion (72–75% of total thymocytes and 22–28% of total splenocytes) of αβ T cells is significantly altered in the thymus and spleen of the Tg mice (Table 2). These thymocytes and splenocytes exhibit a normal expression pattern of CD4 and CD8 glycoproteins

Table 1. Copy number of Tg and relative level of their transcripts in the various Tg mice

| Mice | Copy number of γ Tg | Copy number of δ Tg | $V_{\gamma 4}$ transcripts in Thymocytes | $V_{\gamma 4}$ transcripts in Splenocytes | $V_{\delta 5}$ transcripts in Thymocytes | $V_{\delta 5}$ transcripts in Splenocytes |
|---|---|---|---|---|---|---|
| LM control | | | 0.008 | 0.004 | 0.032 | 0.004 |
| γδ-1355 | 2 | 5 | 0.160 | 0.028 | 0.352 | 0.049 |
| γδ-1314 | 2 | 1 | 0.045 | 0.008 | 0.025 | 0.006 |
| γδ-1313 | 1 | 5 | 0.078 | 0.019 | 0.454 | 0.071 |
| γ-817 | 10 | | 1.091 | 0.943 | | |
| γ-818 | 1 | | 0.276 | 0.292 | | |
| γ-819 | 6 | | 0.433 | 0.468 | | |

The copy numbers of the Tg were estimated from the intensity of the Southern blot bands obtained by hybridizing *Eco*RI-digested liver DNA with the $V_{\gamma 4}$ or $V_{\delta 5}$ probe. A known amount of *Eco*RI-digested pγ-4 and pδ-23/15 cosmid DNA electrophoresed side by side with the liver DNA provided the standard. The γ and δ RNAs were detected by the $V_{\gamma 4}$ and $V_{\delta 5}$ probes, respectively, and their relative content was estimated from the band intensity using the actin RNA as the internal control. Results are expressed in arbitrary units.

Table 2. Surface expression of γδ TCR and αβ TCR on thymocytes and splenocytes of γδ Tg mice

| Mice | Age, weeks | Total thymocytes | Thymocytes ($\times 10^{-6}$) stained with | | | Total splenocytes | Splenocytes ($\times 10^{-6}$) stained with | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Anti-γδ | Anti-KN6 | Anti-αβ | | Anti-γδ | Anti-KN6 | Anti-αβ |
| γδ-1313 | 9 | 79 | 1.26 (1.6) | 1.02 (1.3) | 56.8 (71.9) | 153 | 6.32 (4.1) | 6.27 (4.1) | 43.0 (28.1) |
| LM | 9 | 78 | 0.16 (0.2) | 0.00 (0.0) | 55.9 (71.7) | 167 | 0.66 (0.4) | 0.00 (0.0) | 43.6 (26.1) |
| γδ-1355 | 8 | 100 | 2.66 (2.7) | 2.66 (2.7) | 72.7 (72.7) | 161 | 4.96 (3.1) | 4.83 (3.0) | 36.1 (22.4) |
| LM | 8 | 99 | 0.10 (0.1) | 0.00 (0.0) | 74.2 (74.9) | 152 | 0.89 (0.6) | 0.00 (0.0) | 39.7 (26.1) |

Numbers in parentheses indicate percent. LM, non-Tg littermates.

(data not shown) and, as the αβ T cells in normal mice (9), do not coexpress γδ TCRs at a detectable level (data not shown). These results indicate that a stringent mechanism for restricting the expression of TCR to one of the two types on a given thymocyte or splenic T cell is active in the Tg mice. Furthermore, the results suggest that in these Tg mice, and probably in normal mice, the γδ and αβ T-cell lineages branch out from common precursor T cells before either of the two types of TCR is expressed on the cell surface.

**The γ and δ Tg Persist in the αβ T Cells but Their Transcripts Are Absent.** To investigate why the Tg products are not expressed on the surface of αβ T cells, we purified αβ T cells (and, as a control, γδ T cells) from γδ-1355 mice by using a FACS and analyzed their DNA (Fig. 2*A*) and RNA (Fig. 2*B*). The αβ T-cell population from γδ-1355 mice carries on average 1.1 copy per cell of $V_{\gamma 4}$ gene segments in the germ-line configurations. Therefore, at most 0.9 (i.e., 2.0 copies minus 1.1 copies) copy per cell of the endogenous $V_{\gamma 4}$ gene segment is expected to have rearranged. The observed 2.3 copies per cell of the $V_4J_1C_1$ γ gene (Fig. 2*A*) indicate that at least 1.4 (2.3 minus 0.9) copies per cell of $V_4J_1C_1$ γ can be attributed to the Tg. This is the minimum estimate of the Tg copy number because it is likely that some of the endogenous $V_{\gamma 4}$ gene segments are deleted by rearrangement of the $J_{\gamma 1}$–distal $V_{\gamma 7}$ gene segment to the $J_{\gamma 1}$ or $J_{\gamma 4}$ gene segment (15). Thus, these results confirm that the γ Tg is retained in the majority of αβ T cells.

Fig. 2*A* also shows the analysis of the δ Tg in the γδ-1355 mouse. The αβ T-cell population retains as many copies (about 4) of the $V_{\delta 5}$ gene segment rearranged to $J_{\delta 1}$ as does the γδ T-cell population, which clearly indicates that the δ Tg is retained in the αβ T cells. Analysis of DNA from γδ-1313 and γδ-1314 led to the same conclusion as to the retention of the Tg (data not shown).

$V_{\gamma 4}$- and $V_{\gamma 5}$-specific RNAs were barely detectable in the αβ T cells purified from γδ-1355 splenocytes in contrast to the γδ T cells purified from the same mice (Fig. 2*B*). Virtually the same results were obtained with γδ-1313 and γδ-1314 (data not shown). We confirmed these findings at the level of cloned T cells: none of four randomly chosen αβ T-cell hybridomas prepared from γδ-1355 thymocytes, in contrast to two γδ T-cell hybridomas similarly prepared, contained RNA detectable with either the $V_{\gamma 4}$ or $V_{\delta 5}$ probe, despite their retention of the γ and δ Tg (data not shown). Thus, the expression of the Tg in the αβ T cells of the γδ Tg mice seems to be blocked at the level of RNA.

**A Transcriptional Silencer in the Flanking Region of the γ Gene.** One possible interpretation of the absence of γ transcripts in αβ T cells of the γδ Tg mice, and of normal mice (31), is that the γ gene carries in its flanking region a cis-acting DNA element that down-regulates its own transcription in these cells. To test this possibility *in vivo*, we constructed another set of Tg mice by using a genomic DNA fragment, pγ-L (Fig. 1), that contains essentially the same γ gene as pγ-4 but much less flanking sequence. The copy numbers of the γ Tg and the augmented levels of the γ gene transcripts present in the thymocytes and splenocytes of three founder γ Tg mice, γ-817, γ-818, and γ-819, are summarized in Table 1.

αβ T cells purified from the spleens of these Tg mice, in contrast to those from the γδ Tg mice (Fig. 2*B*), harbored abundant γ RNA of an appropriate size [1.5 kilobases (kb)] detected by the $V_{\gamma 4}$ probe (data not shown). Since the rearrangement of the endogenous $V_4J_1$ γ gene was not inhibited in the αβ T cells of these Tg mice (data not shown), the $V_4$ probe-positive RNA may have been transcribed either from the γ Tg or the endogenously rearranged $V_4J_1$ γ gene or both. To investigate this issue, we prepared αβ T-cell hybridomas

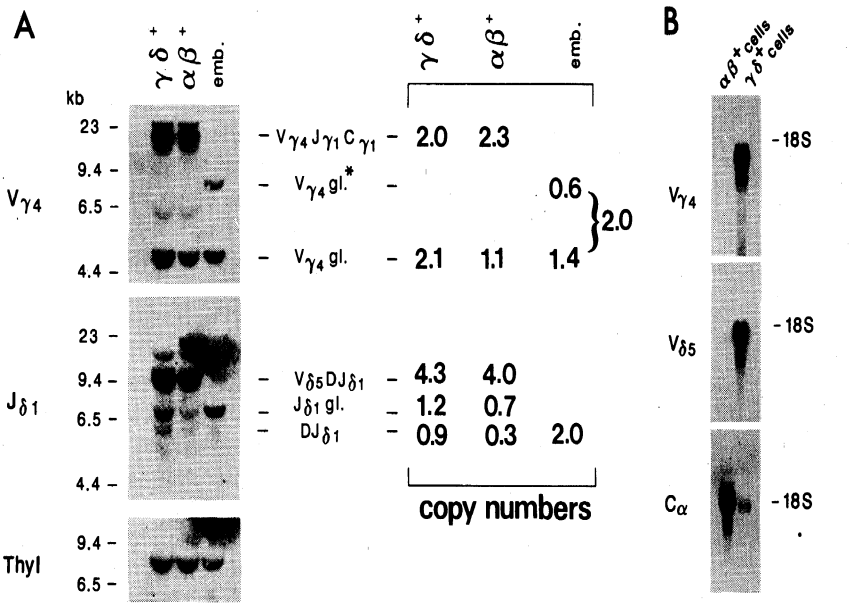


FIG. 2. Southern and Northern blot analyses of nucleic acids from the αβ $TCR^+$ and γδ $TCR^+$ splenic T-cell populations of the Tg mouse γδ-1355. (*A*) DNAs extracted from the purified T-cell subpopulations as well as from C57BL/6 embryos (emb.) were digested with *Eco*RI and analyzed (5 μg per lane) with the $V_{\gamma 4}$, $J_{\delta 1}$, or Thy-I probe. The band assignments were made according to previous studies (12, 30). gl. designates the respective gene segment in the germ-line configuration. $V_{\gamma 4}$ gl.* designates an incompletely digested $V_{\gamma 4}$ gene segment in the germ-line configuration (31). To deduce the copy numbers of the γ and δ genes and their fragments, the relative intensity obtained for each band was normalized for input DNA variation by using the intensity of the Thy-I band in the same lane. The copy number was then calculated by dividing the normalized relative intensity by that of the $V_{\gamma 4}$ or $J_{\delta 1}$ germ-line band of the embryo DNA and then multiplying by 2. (*B*) RNAs extracted from the purified T-cell subpopulations were analyzed (10 μg per lane) with the $V_{\gamma 4}$, $V_{\delta 5}$, or $C_\alpha$ probe. The position of the 18S ribosomal RNA is indicated.

(and, as a control, γδ T hybridomas) from the thymocytes of the γ Tg mice γ-817. As expected, each of these hybridomas, in contrast to the αβ T-cell hybridomas prepared from the γδ Tg mice, contained the $V_{\gamma 4}$ probe-positive, 1.5-kb RNA (data not shown). Also as expected, most of these hybridomas contained, in addition to the γ Tg, an endogenously rearranged $V_4J_1$ γ gene(s) and/or the $V_4J_1$ γ gene derived from the fusion partner BW5147 (15, 32) (Fig. 3*A*).

We prepared cDNA from total RNA extracted from the αβ T hybridomas (as well as from the control γδ T hybridomas) and amplified a 200-base-long segment of the $V_4J_1$ γ cDNA by the PCR (27) using appropriate oligonucleotide primers. The PCR products were incubated with the restriction enzyme *Xba* I and the reaction products were analyzed by agarose gel electrophoresis. Since the unique *Xba* I site is destroyed in the pγ-L Tg (Fig. 1), the amplified DNA corresponding to the Tg will be resistant to *Xba* I digestion, while any amplified DNA derived from endogenous or BW5147-derived $V_4J_1$ γ genes will be split into 150- and 50-base-pair (bp) fragments. As shown in Fig. 3*B*, the three control γδ T-cell hybridomas (nos. 9, 11, and 12) yielded both 200- and 150-bp DNA fragments (the 50-bp DNA fragment, the other product of the *Xba* I digestion, was too short to be visualized clearly in this agarose gel). By contrast, all eight αβ T-cell hybridomas examined yielded only the 200-bp fragment indicative of transcripts from the γ Tg. Essentially the same results were obtained with the other two pγ-L-derived Tg mice, γ-818 and γ-819 (data not shown).

In conclusion, there exist abundant transcripts from the $V_4J_1$ γ Tg in the T-cell hybridomas derived from the αβ thymocytes of the pγ-L Tg mice. In contrast, virtually no transcripts of the endogenously rearranged and/or BW5147-derived $V_4J_1$ γ gene(s) are detected in the same T-cell hybridomas (Fig. 3*B*). Since these results were obtained with three Tg founder mice it is unlikely that the differential expression of the Tg and endogenous (and/or the thymoma derived) γ genes is caused by the specific sequence context in which the Tg are integrated. Thus, these results suggest that the γ gene carries, in its flanking regions, a cis-acting DNA element that down-regulates the amount of γ transcript in αβ T cells. The results further suggest that this silencer element is in the flanking regions present on the pγ-4 clone but absent on the pγ-L clone.

## DISCUSSION

Our finding that αβ T cells are generated in the γδ double Tg mice as abundantly as in non-Tg littermates does not support the Pardoll–Allison model. The αβ T cells retain the Tg but they are apparently transcriptionally silent. The analysis of Tg mice constructed with the short γ gene clone (pγ-L) strongly suggests that the transcription of $V_4J_1$ genes present in αβ T cells is repressed via a DNA element (a silencer) located in its flanking region. In light of these findings we propose a model for the differentiation of αβ and γδ T cells (Fig. 4). The new model, like the Pardoll–Allison model, assumes that γ and δ gene rearrangements occur prior to the completion (i.e., V to DJ joining) of β gene rearrangement and the initiation of α gene rearrangement. Productive rearrangements of both γ and δ genes are, of course, a prerequisite for the generation of γδ thymocytes. However, the failure to rearrange both γ and δ genes productively is not a requirement for the generation of αβ thymocytes. We suggest that the putative machinery acting on the γ silencer is activated in a fraction of immature thymocytes and that it is from these cells that αβ thymocytes are generated. If the activation of the silencer machinery occurs prior to γ and δ gene rearrangements, αβ and γδ T-cell lineages are completely separate and no αβ thymocyte pre-

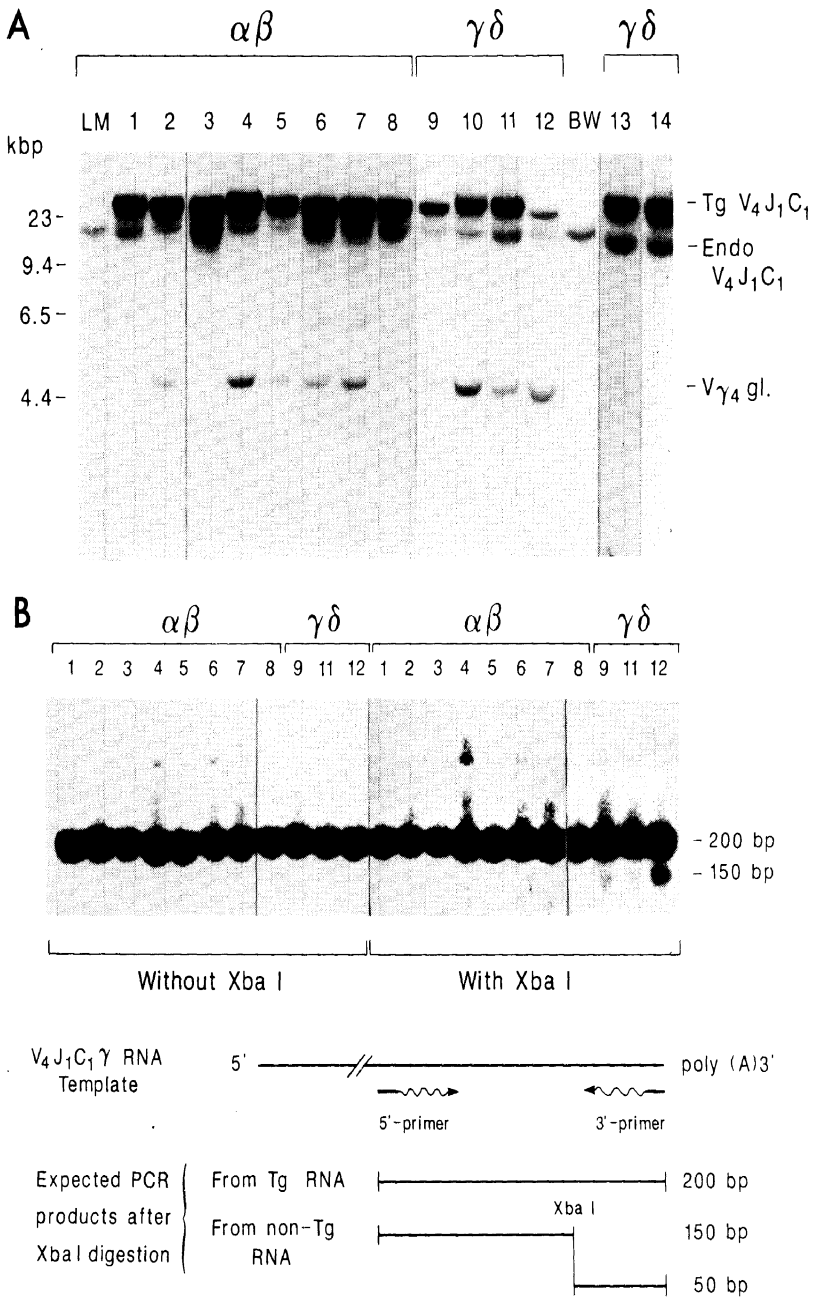


FIG. 3. Southern blot and PCR-aided Southern blot analyses of nucleic acids from T-cell hybridomas prepared from Tg mouse γ-817. (*A*) DNAs extracted from eight (lanes 1–8) γ-817-derived αβ T-cell hybridomas, six (lanes 9–14) γ-817-derived γδ T-cell hybridomas, one (lane LM) normal mouse-derived αβ T-cell hybridoma, and the fusion partner thymoma BW5147 (lane BW) were digested with *Eco*RI and analyzed (5 μg per lane) with the $V_{\gamma 4}$ probe. Since the Tg (10 copies) are concatemerized, they appear as a heterogeneous band (Tg $V_4J_1C_1$) containing large (>30 kb) DNA fragments in contrast to the 16-kb band containing the endogenously rearranged and/or BW5147-derived $V_4J_1C_1$ γ gene. Note that the lack of germ-line $V_{\gamma 4}$ band ($V_{\gamma 4}$ gl.) and/or the augmented intensity of rearranged $V_4J_1C_1$ γ gene band of the non-Tg type (Endo $V_4J_1C_1$) over the intensity of the corresponding band of BW5147 DNA indicate that at least one copy of the endogenous $V_{\gamma 4}$ alleles is rearranged in most hybridomas. (*B*) PCR products of RNA from the eight (lanes 1–8) αβ T-cell hybridomas and three (lanes 9, 11, and 12) γδ T-cell hybridomas, all derived from the Tg mouse γ-817, were analyzed by Southern blotting with and without predigestion with *Xba* I. The 3′ $C_{\gamma 1}$ probe used was the *Hin*dIII/*Pvu* II fragment (700 bp) from clone pγ-L (Fig. 1).

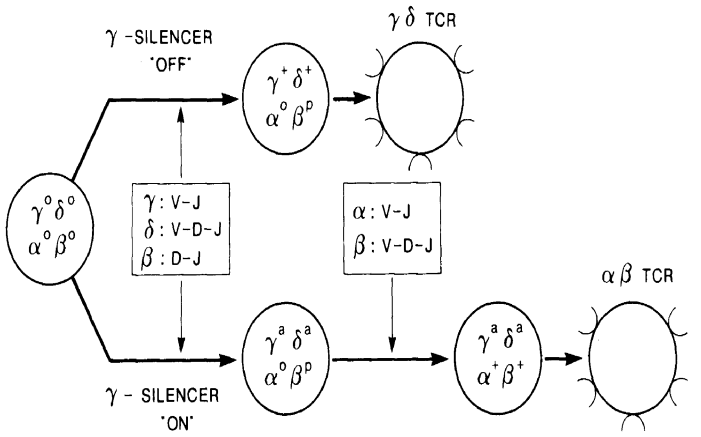


FIG. 4. The silencer model of $\alpha\beta$ and $\gamma\delta$ T-cell differentiation. Superscripts O and + denote that the gene is in the germ-line configuration and productively rearranged, respectively, while superscripts a and p designate that the gene is in any state (i.e., in germ-line configuration, productively or nonproductively rearranged or deleted) and partially rearranged (i.e., D–J joined), respectively. See text for more details.

cursors will ever express $\gamma\delta$ TCRs. We favor this possibility because no $\alpha\beta$ TCR–$\gamma\delta$ TCR double producers are detectable in either normal mice (9) or in the $\gamma\delta$ double Tg mice. However, activation of the machinery could occur simultaneously or shortly after the completion of $\gamma$ and $\delta$ gene rearrangements, in which case precursors of some $\alpha\beta$ T cells may express $\gamma\delta$ TCRs transiently.

It is possible that the generation of $\alpha\beta$ T cells was not blocked in the $\gamma\delta$ Tg mice because the expression of the $\gamma$ or $\delta$ Tg was abnormally delayed in these mice. We argue that this is unlikely because cells bearing Tg-encoded TCRs appear earlier than $\alpha\beta$ thymocytes in developing thymuses (unpublished results). Furthermore, in the $\gamma\delta$ T cells of the Tg mice $\gamma\delta$-1355, $V_{\gamma4} \rightarrow J_{\gamma1}$ and $V_{\delta} \rightarrow DJ_{\delta1}$ rearrangements are completely inhibited (Fig. 2*A*) by allelic exclusion (18) and virtually all $\gamma\delta$ TCRs expressed are Tg-encoded (Table 2). Should the surface expression of the Tg-encoded $\gamma\delta$ TCR be abnormally delayed in the $\gamma\delta$ Tg mice, the endogenous $\gamma$ and $\delta$ genes would have rearranged and been expressed on the surface of $\gamma\delta$ T cells.

It should be noted that the present study deals with the major $\gamma\delta$ T-cell subset expressing the $C_{\gamma1}$-associated $\gamma$ genes and does not directly address the lineage relationship involving the $C_{\gamma4}$-expressing $\gamma\delta$ T cells. However, since the latter $\gamma$ genes are also repressed in $\alpha\beta$ T cells (Y. Takagaki and S.T., unpublished data) we suspect that a similar mechanism applies to this $\gamma\delta$ T-cell subset. In contrast, the $V_2J_2C_2$ $\gamma$ gene utilizing the third $C_\gamma$ gene segment is not usually repressed in $\alpha\beta$ T cells (1, 2, 31). However, no freshly isolated T cell has ever been shown to express the product of these $\gamma$ genes on the cell surface.

Recently, the issue of whether $\alpha\beta$ and $\gamma\delta$ T-cell lineages are completely separate was addressed by analysis of extrachromosomal circular DNA generated by $V_\alpha$–$J_\alpha$ joinings, but two groups came up with opposite conclusions (33, 34). We note that the $\gamma$ silencer model presented here would make more sense if the $\delta$ gene rearrangement is not entirely repressed in the precursors of $\alpha\beta$ T cells.

The present study was not designed to determine the precise location of the putative $\gamma$ silencer element. However, we can conclude that it is outside of the sequences contained in the 15-kb pγ-L clone. The finding that the $\gamma$ Tg is repressed in $\alpha\beta$ T cells of the $\gamma\delta$ Tg mice suggests that the silencer is on the 40-kb pγ-4 clone. However, since these Tg mice were made by coinjection of the $\gamma$ and $\delta$ gene clones and coinjected DNAs are known to be cointegrated, it is possible that the $\gamma$ Tg is repressed by a $\delta$ gene-associated silencer. We argue that this is unlikely because the $\delta$ gene, at least in the 43-kb construct used, does not seem to be transcriptionally silent in an $\alpha\beta$ T-cell clone (28).

Finally, the silencer model of T-cell development is supported by our recent finding that the generation of $\alpha\beta$ T cells is severely blocked in Tg mice carrying $\gamma$ and $\delta$ Tg without the associated silencer (35).

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