

TRANSIENT REARRANGEMENTS OF THE T CELL ANTIGEN RECEPTOR α LOCUS IN EARLY THYMOCYTES

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The thymus is the major differentiative organ for T lymphocytes in higher vertebrates. T cell precursors arise in the bone marrow and spleen from hematopoietic stem cells, migrate to the thymus and there undergo a series of maturational and proliferative steps that result in the production of functional T lymphocytes. Although much of this process remains mysterious, the major thymic precursor compartment has been identified as the dull Ly-1 (dLy-1)/double-negative population, and has the surface phenotype: Thy-1⁺, Ly-1^{dull}, Lyl-2⁻, L3T4⁻ (1, 2). These cells make up 3–5% of the adult thymus and are capable of repopulating irradiated mice with mature T cells (2). They are distinct from bone marrow stem cells in that they have limited regenerating capacity, but they are able to generate all major cell types in the thymus, namely the double-positive cells (Thy-1⁺, Ly-1⁺⁺, Lyl-2⁺, L3T4⁺), which make up the largest subpopulation (75%) in the adult thymus, and the single-positive cells (Thy-1⁺, Lyl-2⁺, L3T4⁻ or Thy-1⁺, Lyl-2⁻, L3T4⁺) (2).

The availability of gene probes for the antigen receptors of T cells (TCR), α , β , and γ (3–8) has reinforced this view of the dLy-1 cells as the earliest T cell subpopulation. Analysis with TCR probes has indicated that while adult dLy-1/double negative thymocytes have β and γ chain mRNA, they have very little or no α mRNA (9, 10). Indications of a sequential expression of TCR genes is also borne out by analyses of fetal thymocytes, where β and γ chain mRNA is expressed several days before α chain mRNA (9, 11–13). This progression of γ and β first, then α , is reminiscent of immunoglobulin heavy chain genes, which are transcribed and translated well before the light chain genes (14).

We have previously investigated the β gene rearrangements in T cell hybridomas made from adult dLy-1 cells (10) and found that one-third of them have only the germline configuration of the β chain gene, consistent with rearrangement and expression of the β locus being an ongoing process in this compartment of cells. In this manuscript we extend our study of the dLy-1 compartment and later stages of T cell differentiation in adult mice to include α and γ chain gene

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rearrangements, and to distinguish between $D_\beta \rightarrow J_\beta$ and presumptive $V_\beta \rightarrow D_\beta J_\beta$ recombination in the dLy-1 hybridomas. In the course of this work, we have discovered a novel series of rearrangements in the locus of many dLy-1 cells and hybrids that occurs 80 kb 5' of the C_α coding region and specifically excludes any of the J_α sequences localized to date. These 5' C_α rearrangements are transient in that while they predominate in cells from the dLy-1 compartment, they are largely absent in splenic T cells and in mature T cell lines or hybrids. This prior rearrangement of the α locus and its apparent coordination with β and γ chain rearrangements in adult dLy-1 cells provides a new phenotype with which to analyze early T cells, and has interesting implications for the regulation of T cell receptor gene expression during thymic differentiation.

Materials and Methods

Preparation of DNA. T cell lines and hybridomas were cultured in RPMI 1640 with 10% FCS. dLy-1 hybridomas are fusion products of dLy-1 thymocytes and BW5147 (10). Nuclei were prepared as previously described (15). To prepare DNA in agarose blocks for subsequent restriction digestions, a modification of the procedure by Bernards et al. (16) was used. The nuclei were mixed with an equal volume of 1% low-melting-point agarose in resuspension buffer (10 mM NaCl, 10 mM Tris HCl, pH 7.5, 25 mM EDTA) and 60 μ g/ml of proteinase K. The suspension was transferred in 0.1-ml aliquots to 96-well microtiter dishes and allowed to solidify. Each agarose block was then incubated for 16–24 h in RSB with 1% SDS at 50°C. After lysis, half an agarose block was washed in 200 volumes of 10 mM Tris, pH 7.5, 10 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), followed by equilibration in 100 volumes of the appropriate restriction buffer. The agarose block was then placed in 50 μ l of restriction buffer with 30 U of restriction enzyme, and digested overnight. The restriction buffer was removed and the agarose was melted at 68°C. After brief incubation at 37°C, another 15–20 U of enzyme was added, as well as spermidine to a final concentration of 2 mM, and the digestion was continued for an additional 2 h. dLy-1 thymocytes were prepared from 6–8-wk-old C57BL/6 mice as previously described (2). The final yield was 0.5% and the cells were 97% Lyt-2^- and L3T4^- , as assessed by staining with appropriate antibodies and FACS analysis. With low cell yields, DNA was prepared from whole cells as described (16). No difference in the quality of the DNA was seen when DNA was prepared from whole cells as opposed to nuclei. Concatamers of λ DNA used as size markers were prepared as described by Carle et al. (17).

Gel Electrophoresis and Southern Blot Analysis. For conventional gel electrophoresis, digested DNA was electrophoresed through 0.7% agarose gels. For pulsed-field gels, electrophoresis was performed in 1% agarose gels in 0.5 \times TBE (1 \times TBE is 89 mM Tris base, 89 mM boric acid, 8 mM EDTA), as described (17). The agarose block containing digested DNA was pipetted into the slots of the gel after melting at 68°C. The electrophoresis apparatus used was built in the Stanford Genetics Department shop according to the specifications published by Carle and Olson (17). The gels were run in 0.5 \times TBE at 10–11°C for 10 h at 300 V. The pulse duration used was 10 s, which gives good separation from 50 to 350 kb. The gels were irradiated at 254 nm for 2 min after ethidium bromide staining, and DNA was transferred to nylon membranes (Gene Screen; New England Nuclear, Boston, MA) according to manufacturer's directions. Probes were generated by hexamer labeling (18) and filters were washed twice for 30 min in 2 \times SSPE (2 \times SSPE is 0.3 M NaCl, 0.2 M NaH_2PO_4 , 0.002 M EDTA, pH 7.4), 0.1% SDS at 55°C, followed by 30 min in 0.2 \times SSPE at 55°C.

Densitometry was performed on an RFT Scanning Densitometer (Transidyne General Corp., Ann Arbor, MI). Graphs were cut out and the surface area was determined by weighing.

Results

α Chain Gene Rearrangements Detected by Pulsed-field Gel Electrophoresis. Studies of α chain gene rearrangement have been hampered by the unusual genomic organization of this gene (19–21). The J_α regions are not clustered but spread out over at least 60 kb of DNA 5' to the C_α region. Our approach to this problem has been to use the recently described technology of pulsed-field gel electrophoresis (17, 22) and rare-cutting restriction enzymes to generate unique DNA fragments large enough to include both C_α and all the J_α coding regions. Fig. 1A shows an ethidium bromide-stained pulsed-field gel of Xho I-digested DNA from dLy-1 hybridomas, using λ DNA concatamers as size markers. The lowest band in the marker lane represents unligated λ DNA of 49 kb, the second, 98 kb, and so forth. Xho I is a useful enzyme for analyzing the C_α locus because there are two Xho I sites just 3' of the C_α 3' untranslated region (23) and they can be cut completely in all the DNA samples we have tested. When blotted and hybridized with a C_α containing probe, C57BL/6 liver DNA displays a major hybridizing band at 120 kb (Fig. 1B). This fragment extends almost entirely 5' of the C_α coding region, as it does not hybridize to a genomic probe just 3' of the Xho I sites adjacent to C_α , and thus includes all 20 J_α sequences that have been identified and mapped from 21 different cDNA clones (19, 20) (Chien, Wallich, and Ivars, unpublished data). Another band is seen at 50 kb and represents a partially methylated Xho I site 5' of C_α . 10 of the 12 original dLy-1 hybridomas (10) were available for analysis by pulse-field gel electrophoresis. Of these, four dLy-1 hybridomas (2E5, 1G10, 1G8, and 2C9) have a band similar to the germline band at 120 kb, in addition to the BW5147-attributable band at 55 kb. 1B5 and 2C10 only show BW5147-derived bands, indicating a deletion at the α locus. Interestingly, these same two hybridomas also appear to have lost the C57BL/6-derived γ chain genes as well, but not those for β (see later sections). The hybridomas 1G5, 3B10, 1D5, and 2D9 have new bands not seen in C57BL/6 liver DNA or BW5147 DNA, suggesting α locus rearrangements. Alterations from the liver DNA pattern are also apparent in an Xho I digest of freshly prepared adult dLy-1 cells, which displays a smear between the 100 and 120 kb bands (Fig. 2A). This is in contrast to that observed for the unfractionated thymocyte DNA, which shows very faint bands at 120 and 100 kb and a continuous smear extending lower than 50 kb. As a control for the intactness of the DNA samples, a probe 3' of the Xho I sites adjacent to C_α was used to hybridize the same filters. As shown in Fig. 2B, sharp bands of similar intensity are present in all lanes. Thus, the pattern seen in the total thymocyte DNA is consistent with multiple rearrangements involving the J_α region and different V_α sequences. This interpretation is supported by estimates that the majority of the thymocytes express α/β heterodimers (24). However, the pattern in dLy-1 cells and hybrids suggests alterations of a very different nature.

Localization of α Chain Gene Rearrangements. Because Xho I digestion appears sensitive to DNA methylation in eucaryotic DNA (the cytosine in the CG pair [25]), it is important to determine whether the altered C_α hybridizing patterns that we observed in dLy-1 cells and hybridomas could be due to differences in the methylation pattern of the α locus with respect to liver DNA. We therefore isolated a series of cosmid clones covering this region by using a fragment

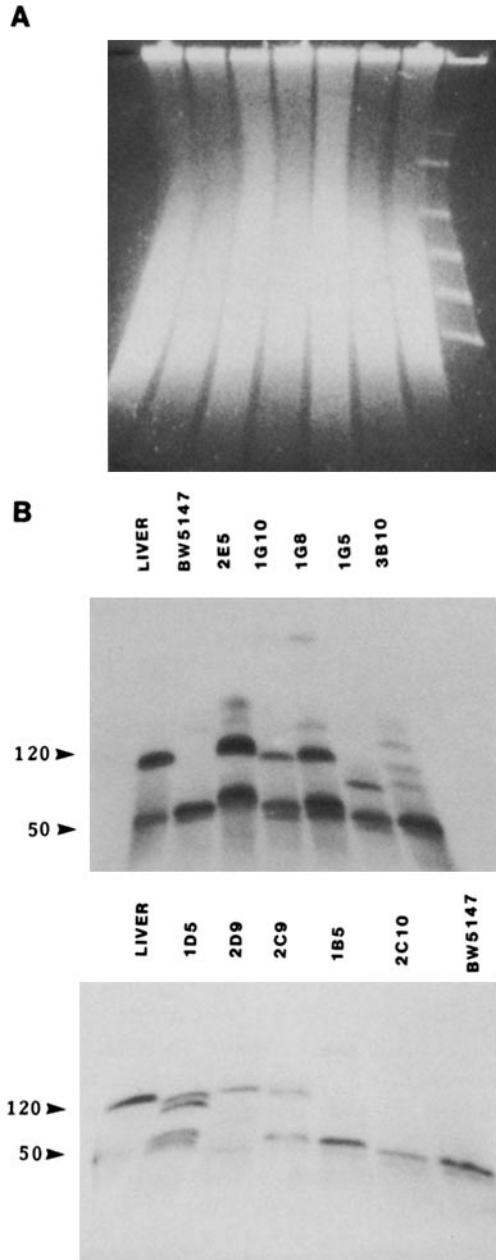


FIGURE 1. α Chain gene rearrangement detected by pulsed-field gel electrophoresis. **A.** An ethidium bromide-stained pulsed-field gel. The gel was run for 10 h at 300 V with a 10-s pulse duration. λ DNA concatamers ranging from 49 to 294 kb were used as size markers. Other lanes show Xho I-digested liver and hybridoma DNAs. **B.** DNA from liver and dLy-1 hybridomas was digested with Xho I and separated by pulsed-field gel electrophoresis. Southern blots were prepared as described and probed with a C_α region genomic probe (probe *D* in Fig. 3A).

(fragment *C*, Fig. 3A) containing the helper hybridoma 2B4 J_α sequence, which is located 55 kb 5' to the C_α (R. Wallich, Y. Chien, and M. Davis, manuscript in preparation). By "walking" further 5' to this *J* region, we obtained cosmid clones extending 110 kb 5' to the C_α region (26), as indicated in Fig. 3A, together with a partial restriction map. Xho I sites are located at 50, 95, 110, and 120 kb away from the sites on the 3' side of C_α . The presence of the first and last of these

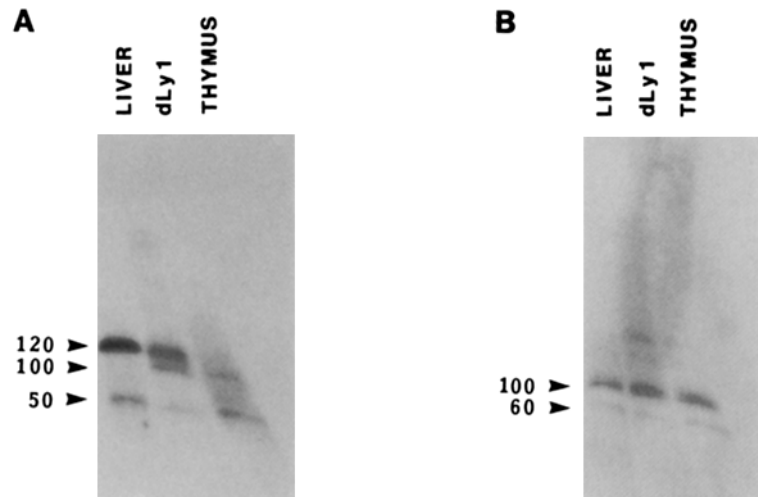


FIGURE 2. Altered configuration of the α chain gene in dLy-1 thymocytes and total thymocytes detected by pulsed-field gel electrophoresis. DNA from liver, dLy-1, and total thymocytes was digested with Xho I and separated by pulsed-field gel electrophoresis. *A*. Southern blot probed with a C_{α} region probe (probe *D* in Fig. 3). *B*. The same blot as in *A* was reprobed with a Bgl I-Bam HI fragment 3' to the C_{α} coding region (probe *E* in Fig. 3A).

sites is in agreement with the sizes of the fragments found in pulsed-field gel studies of liver DNA (e.g., the 120 and 50 kb bands). dLy-1 thymocyte and hybridoma DNAs were digested with Bam HI and probed with a fragment spanning the 95 kb Xho I site (Fig. 3A, fragment A). The results are shown in Fig. 3B. In the dLy-1 thymocyte DNA sample, many hybridizing bands in addition to the germline band were observed. All the dLy-1 hybridomas except 1G10 and 2C9 show hybridizing bands other than that of germline, and the sizes are similar to those observed in dLy-1 cell population. Because BW5147 has deleted this region (Fig. 3B), the new bands seen in the hybridomas must arise from the dLy-1 thymocytes. These results indicate that rearrangements have occurred ~ 80 kb 5' to the C_{α} (or ~ 90 kb 5' to the Xho I sites located 3' to C_{α}) in dLy-1 thymocytes, and are represented clonally in the dLy-1 hybridomas that, in most cases, have no germline material at all from this region. Similar evidence of rearrangement was detected with other enzymes that are not sensitive to CG methylation (such as Eco RI and Sac I digestions, data not shown).

In contrast to the results with probe A, all the dLy-1 hybridoma DNAs show a germline configuration when probed with a fragment just 3' to probe A (probe B, data shown in Fig. 3C), and the intensity of the germline band in dLy-1 DNA is undiminished when compared with that from the same amount of liver DNA. Similar results were obtained by using probe C, further 3' and including the 2B4 J_{α} gene segment (Fig. 3D). This indicates that the rearrangements we see in the α locus of dLy-1 cells specifically affects the region covered by probe A but does not, for example, move it closer to the C_{α} coding region. This would appear to rule out the involvement of any of the J_{α} s found expressed in mRNAs from either thymocytes or mature T cell lines and hybrids, all of which have been mapped in the region from 1 to 60 kb 5' of C_{α} (as indicated in Fig. 3A [19,

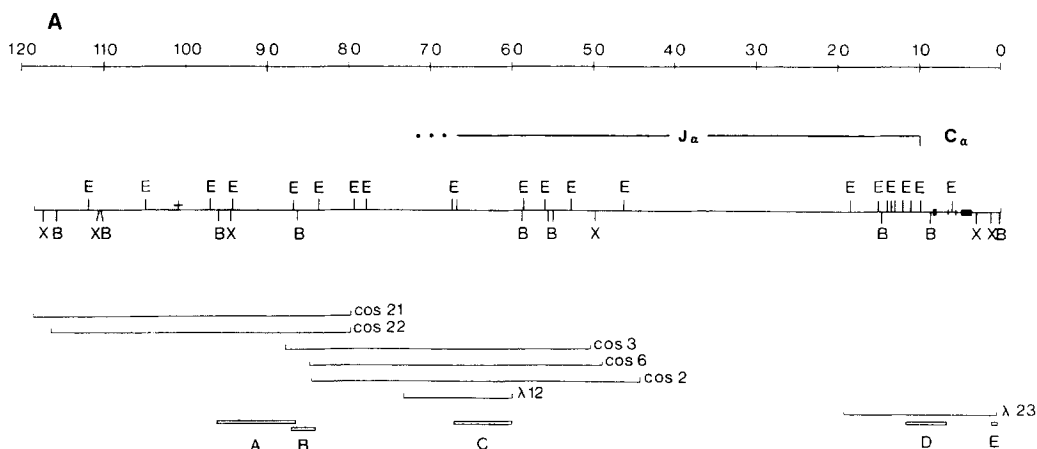


FIGURE 3. A. Genomic organization of the α chain locus and the locations of the probes. A series of cosmid clones extending 110 kb 5' to C_α region were isolated by using probe C, which contains the helper hybridoma 2B4 J_α sequence, located 65 kb 5' to the C_α (26). Restriction sites are given for Bam HI (B), Eco RI (E), and Xho I (X). Unassigned sites between restriction sites are indicated by +. The solid line labeled J_α indicates the location of J_α region genes (19, 20, and Chien, Wallich, and Ivars, unpublished data). The dotted line indicates the possible presence of additional J_α region genes. Boxes indicate the probes used in this paper. B. α Chain rearrangement 80 kb 5' to the C_α region in dLy-1 thymocytes and dLy-1 hybridomas. Probe A was used to analyze a Southern blot of Bam HI-digested DNA from liver, dLy-1 thymocytes, dLy-1 hybridomas, and BW5147 DNA, as indicated. C. The rearrangement 80 kb 5' to the C_α region does not involve the majority of J_α region genes. DNA from liver, dLy-1 thymocytes, and dLy-1 hybridomas were digested with Eco RI and probed with probe B. All the DNA samples display two bands, which are different from the restriction map of the cosmid clones. This appears due to the presence of a restriction fragment length polymorphism between BALB/c (the strain used for the cosmid library) and C57BL/6. D. The same Southern blots as in 3C were probed with probe C.

20]). Thus, the changes in the α locus observed in dLy-1 thymocytes seems to be an example of some hitherto undescribed type of rearrangement in the α locus and may represent a precursor to functional $V_\alpha \rightarrow J_\alpha$ joining.

Absence of 5' C_α Rearrangements in Mature T Cell Lines. That this type of rearrangement is absent in many mature T cell lines is indicated in Fig. 4, where four helper T cell lines and one cytotoxic T cell line are surveyed for hybridization with probe A. As shown in this figure, all of these lines have deleted the 5' C_α region from both chromosomes. A parallel survey of four functional T cell hybridomas indicates that all but one (2B4 [26]) have also deleted this region (data not shown). These findings are consistent with what one would expect if rearrangements 5' of C_α were subsequently deleted by $V_\alpha \rightarrow J_\alpha$ joinings. That rearrangements 5' of C_α are T cell-specific and not some general instability is indicated by the fact that two different B-lineage-derived tumor lines and a fibroblast line (L cells) are all germline with respect to this portion of the α chain locus (Fig. 4).

α Chain Gene Rearrangement. Previously we reported that 8 of 12 dLy-1 hybridomas showed β chain gene rearrangements (10). Repeated passage of these lines have not altered these original patterns, one indication of the stability with respect to rearrangement of BW5147 fusions. We have further characterized the β chain rearrangements in these hybridomas with $D_{\beta 1}$ - and $D_{\beta 2}$ -specific probes

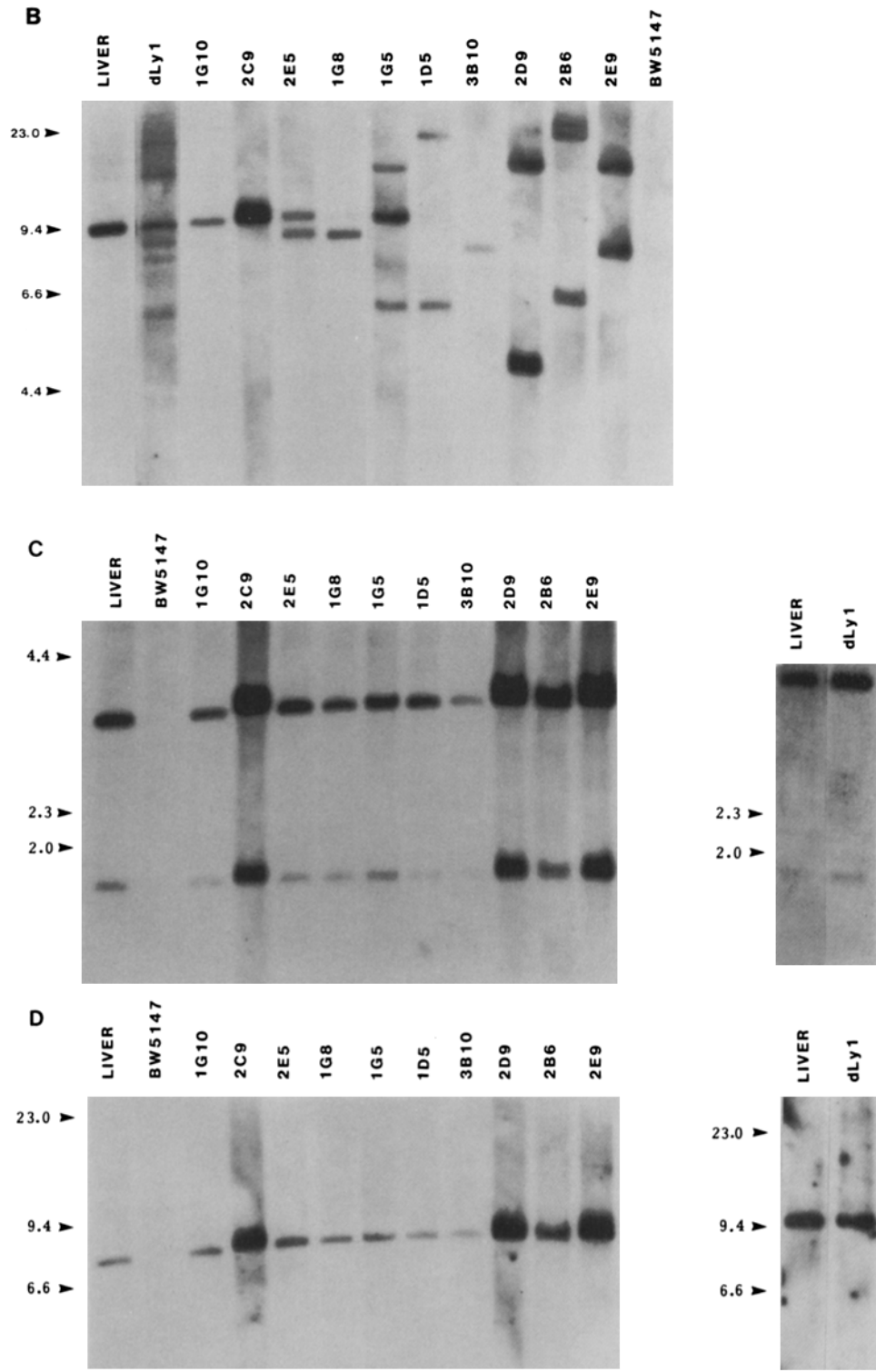


FIGURE 3. B-D

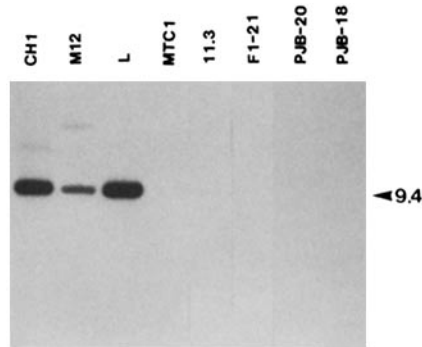


FIGURE 4. Probe A from Fig. 3A was used to analyze cell line DNAs, which include two B cell lymphoma lines (CH1 and M12); a fibroblast line (L cells); a cytotoxic T cell line (MTC1 [36]); one whale myoglobin-reactive helper cell line (11.3; A. Livingstone and G. Fathman, manuscript in preparation); and three myelin basic protein-reactive helper cell lines (F1-21, PJB-20, and PJB-18 [37]). The faint bands in the CH1 and M12 lanes are due to partial digestion products.

in order to determine which are the result of DJ joining and which represent VDJ rearrangements. The $D_{\beta 1}$ and $D_{\beta 2}$ region hybridizations and the location of the probes is indicated in Fig. 5. If VDJ joining has occurred, these sequences would be deleted, except in the case of an inversion such as occurs with $V_{\beta 14}$ (27), but this is the only V_{β} known to be located 3' of C_{β} (T. Lindsten, N. Lee, and M. Davis, 1987. *Proc. Natl. Acad. Sci. USA*. In press.) and is not rearranged in any of these hybridomas (data not shown). As the fusion partner, BW5147 has undergone VDJ rearrangements on both alleles (N. Lee and M. Davis, manuscript in preparation), any rearrangements seen with D_{β} region-specific probes can be attributed to the chromosomes contributed by the dLy-1 thymocytes. Fig. 5 shows Southern blots of Eco RI-digested hybridoma and liver DNAs probed with the $D_{\beta 1}$ and $D_{\beta 2}$ probes, and the interpretation of this data is summarized in Table I together with the 5' C_{α} rearrangements discussed in the previous section, and an analysis of γ chain gene rearrangements discussed in the following section. Rearrangements designated "other" to $C_{\beta 1}$ or $C_{\beta 2}$ presumably represent VDJ joining events, but one cannot exclude possible D \rightarrow D rearrangements or D \rightarrow J joinings involving D regions other than $D_{\beta 1}$ or $D_{\beta 2}$. All hybrids were analyzed for C_{β} rearrangements using the enzymes Hpa I and Pvu II (10 and this manuscript, data not shown). Despite a possible underestimate of $D_{\beta} \rightarrow J_{\beta}$ joining events, it is interesting that three of the eight β chain rearranged hybrids have only $D_{\beta} \rightarrow J_{\beta}$ rearrangements (1B5, 1G5, and 2B6, Fig. 5 and Table I); the former of these has a germline and a rearranged chromosome for all three loci (α , β , and γ ; see next section). This data is consistent with $D_{\beta} \rightarrow J_{\beta}$ being the initial event in gene formation as has been shown in fetal thymocyte hybridomas and fetal thymocytes (28, 29) but shows that this form does not predominate in adult dLy-1 cells as a population.

To determine whether the pattern of β chain gene rearrangement seen in this panel of hybridomas is representative of normal cells, we also analyzed β chain gene rearrangement in the dLy-1 thymocyte population. Fig. 5C shows a Southern blot of Hpa I-digested C57BL/6 liver DNA and dLy-1 thymocyte DNA, probed with a C_{β} cDNA (86T5) probe (3). Rearrangements in the dLy-1 thymocyte DNA are evident from a series of bands representing D \rightarrow J joinings. The smear of bands at 16–17 kb may represent $D_{\beta 2} \rightarrow J_{\beta 2}$ joinings. $D_{\beta 1} \rightarrow J_{\beta 1}$ joinings appear evident, as a set of bands ranging in size from 11.0 to 9.2 kb and $D_{\beta 1} \rightarrow J_{\beta 2}$ joinings are present as bands of 7.9–6.8 kb. Densitometry indicates

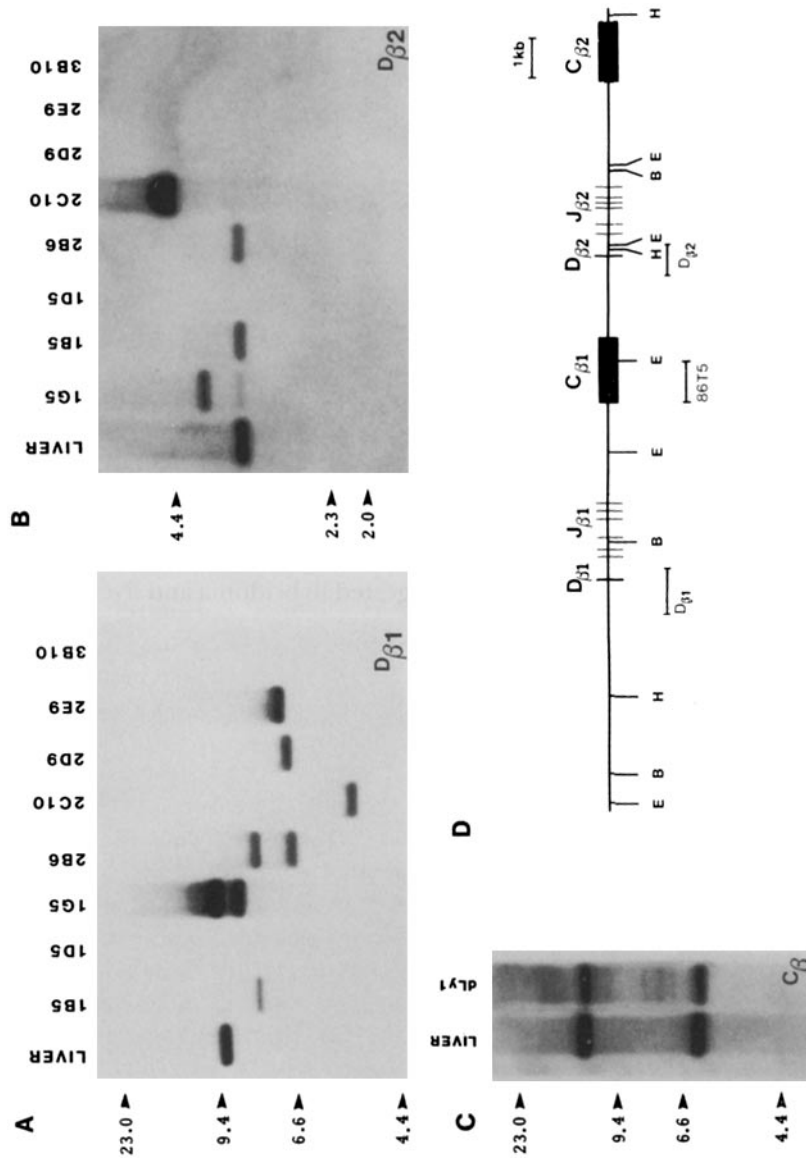


FIGURE 5. β Chain rearrangement in dLy-1 hybridomas and dLy-1 thymocytes. A. Southern blot of Eco RI-digested DNA from liver and dLy-1 thymocytes was probed with the 86T5 cDNA clone (3). B. Southern blot of Eco RI-digested DNA from liver and dLy-1 hybridomas was probed with a $D_{\beta 1}$ -specific probe. C. Southern blot of Bam HI-digested DNA from liver and dLy-1 hybridomas was probed with a $D_{\beta 2}$ -specific probe. D. Southern blot of Eco RI-digested DNA from liver and dLy-1 thymocytes was probed with the 86T5 cDNA clone (3). E. Restriction map of the C_{β} gene locus and the locations of probes. Restriction sites are given for Bam HI (B), Eco RI (E), and Hpa I (H).

TABLE I
5' C α , β , and γ Chain Gene Rearrangements in Double-negative Hybridomas

	5' C α		β					γ			
	Germline	Rearranged	Germline	D β 1-J β 1	D β 1-J β 2	D β 2-J β 2	Other to C β 1	Other to C β 2	Germline	V γ 1C γ	V γ 2C γ
1G10	+		+						+		
2C9	+		+						+		
2E5	+	+	+						+		
1G8	+	+	+						+	+	
1G5	+	+	+	+		+			+	+	
1D5		+							++*	+	
3B10		+							++	+	+
2D9		+			+				+	X [‡]	Y
2B6		+		+	+					+	+
2E9		+			+				+	+	+
1B5	0 [‡]	0		+					0	0	0
2C10	0	0				+	+		0	0	0

* In the β locus, both 1D5 and 3B10 have two distinct rearrangements to C β 2, as indicated by ++.

‡ In the γ chain locus, the hybridoma 2D9 has V γ 1 and V γ 2 rearrangements, but they are distinct from the normal V γ 1C γ 1 and V γ 2C γ 2 pattern, as indicated by X and Y.

‡ 0 indicates the locus has been deleted.

that 50% of the C β 1 germline band (12 kb) and 56% of the C β 2 germline band (6 kb) is present in the dLy-1 thymocyte DNA as compared to liver DNA. Thus, at least half of the dLy-1 thymocytes have undergone β chain gene rearrangements, in reasonable agreement with the analysis of the hybridomas.

γ Gene Rearrangement. The dLy-1 hybridomas were also analyzed for γ chain gene rearrangements (Fig. 6, and summarized in Table I). Because the fusion partner BW5147 also has γ gene rearrangements, it is necessary to find a restriction enzyme that will distinguish C57BL/6 γ gene rearrangements from those of the AKR strain, from which BW5147 derives. One such restriction enzyme is Bam HI. Fig. 6 shows a Southern blot of Bam HI-digested DNA from the dLy-1 hybridomas probed with a C γ 2 cDNA probe (8) (courtesy of Drs. T. Yokota and K. Arai). Germline bands from mice of the same inbred strain (C57BL/6) are present at 12.0, 10.5, 9.0, 8.0 and 3.3 kb. Five hybridomas, 1D5, 3B10, 2D9, 2B6, and 2E9, showed a deletion of the 9.0-kb germline band, and all except 2D9 displayed a new band at 13.5 kb; 2D9 had a new band at 4.9 kb. The hybridomas 1G5 and 1G8 showed a faint band at 13.5 kb, as well as the germline band at 9.0 kb, indicating rearrangement of one C γ allele. As mentioned previously, the figure also shows that 1B5 and 2C10 only have the BW5147 pattern, and therefore appear to have deleted the C57BL/6 γ genes. Southern blots of Bam HI-digested hybridoma DNA probed with a V γ 1 cDNA probe showed that V γ 1 and C γ resided on the same 13.5 kb rearranged band in 1D5, 3B10, 2B6, 2E9, 1G5, and 1G8 (Fig. 6B). In the case of 2D9, the V γ rearrangement was not overlapping with the C γ rearrangement, as a new band was seen at 5.4 kb. With a probe for V γ 2 (30-32) (referred to as V γ 1 in Garman et al. [30]), the other major V γ species in mice shown to be expressed in dLy-1 thymocytes (30), four of the seven hybridomas that have C γ rearrangements were also rearranged (Table I). In summary, 7 out of 10 of the dLy-1 hybridomas displayed C γ gene rearrangements, and all of these involved V γ 1. Many had rearranged V γ 2 as well. No hybrid that had a γ chain gene rearrangement lacked a 5' C α

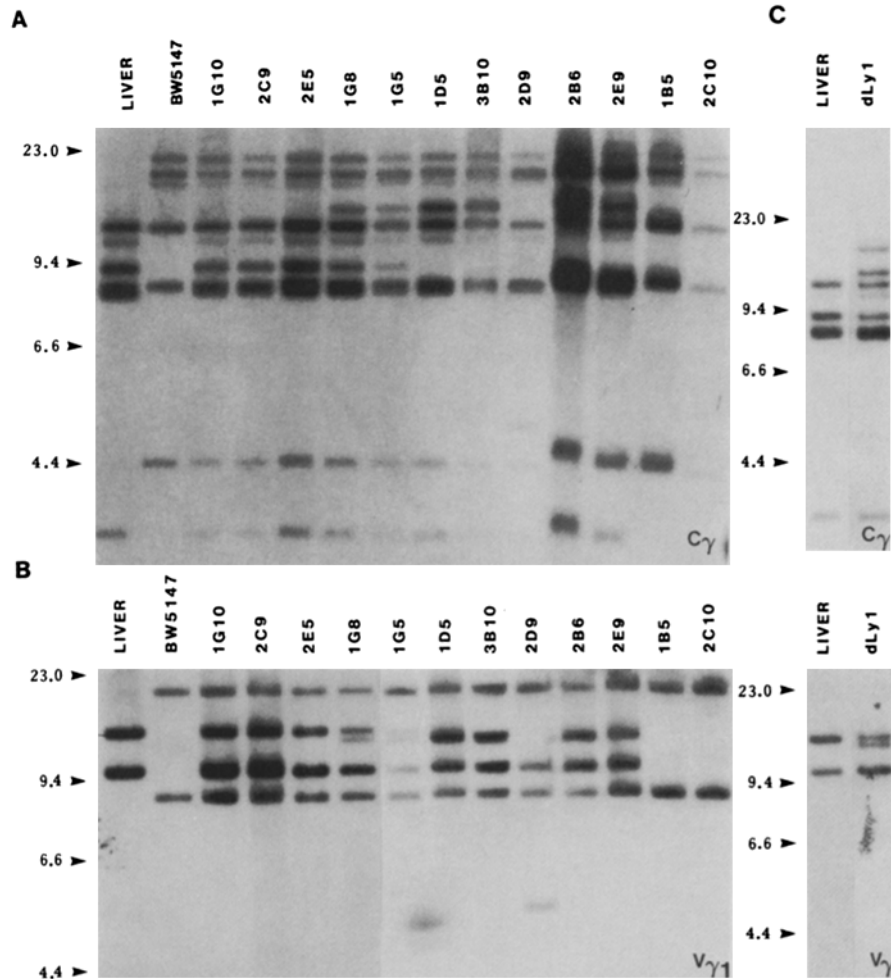


FIGURE 6. γ Gene rearrangement in dLy-1 hybridomas and dLy-1 thymocytes. Southern blots of DNA from liver and dLy-1 hybridomas digested with Bam HI were probed with: A, a C_{γ} probe; B, a $V_{\gamma 1}$ probe; C, liver and dLy-1 thymocyte DNA digested with Bam HI and probed with a C_{γ} -containing cDNA clone.

rearrangement, and all but one (1G8) had β chain gene rearrangements (Table I).

The γ gene rearrangements seen in the dLy-1 thymocyte population are consistent with those found in the hybridomas. Fig. 6C shows a Southern blot of Bam HI-digested C57BL/6 liver, and dLy-1 thymocyte DNA probed with a C_{γ} cDNA probe. In addition to the 13.5 kb band, a second band is seen at 17.0 kb in the dLy-1 thymocyte DNA. This band overlaps with the BW5147 rearrangement in the dLy-1 hybridomas. The $V_{\gamma 1}$ cDNA probe only shows hybridization to the 13.5 kb band, whereas the $V_{\gamma 2}$ probe hybridizes to the 17.0 kb band (data not shown). Densitometry revealed that 55% of the 12.0 kb C_{γ} germline band and 40% of the 9.0 kb C_{γ} germline band are present in the dLy-1 thymocyte

DNA as compared to liver DNA. Thus, at least 60% of the dLy-1 thymocytes have undergone V_γ rearrangements.

Discussion

TCR Gene Rearrangements in the Thymus. The data presented here add to the emerging consensus that the thymus is the major lymphoid organ in which TCR genes are first rearranged and expressed (33). This is indicated by the fact that we can separate the dLy-1 hybridomas described here into distinct categories, as summarized in Table I. These categories include hybrids with no detectable rearrangements (such as 1G10 and 2C9) and others with 5' C_α , DJ, and probable VDJ $_\beta$ and VJ $_\gamma$ chain gene rearrangements. The data in Table I also suggests that there may be a hierarchy of rearrangement within this rearrangement phase, such as: 5' C_α before γ , and γ before β . Together with the data of Born et al. (28, 29), which indicates a progressive appearance of γ and β chain gene rearranged hybridomas during fetal development in the thymus, our data suggest that there is an ordered progression of T cell receptor locus rearrangements in the thymus. This specific order of rearrangement may relate to feedback control mechanisms similar to those thought to be operating in the immunoglobulin gene systems (34).

Possible Significance of the Rearrangements 80 kb 5' of C_α . The rearrangements that we see 80 kb 5' of C_α are not readily ascribable to any known type of TCR or immunoglobulin rearrangement. We conclude this for two reasons: (a) There is very little or no steady-state C_α -containing mRNA in rigorously purified dLy-1 cells (10), the low levels of α message seen in some studies (9) being attributable to Ly-1-bright double-negative cells that are now known to express α chain mRNA (R. Budd, personal communication). Constant region-containing transcripts are a characteristic of most types of antigen receptor gene rearrangement (and some pre-rearrangement states, as recently reviewed by Yancopoulos and Alt [35]). (b) The 5' C_α rearrangements specifically exclude any of the J_α gene segments localized to date (19, 20; Chien, Ivars, and Wallich, unpublished data). This phenomenon seems relatively cell type- and stage-specific, as this portion of the α locus is deleted in most mature T cells and is in the germline configuration in several B cell tumor lines and in L cells (see Results). Although we cannot be sure that these rearrangements are necessary precursors to functional $V_\alpha \rightarrow J_\alpha$ joining and mRNA production, their predominance among the dLy-1 cells and hybridomas as well as in fetal thymocytes (26) makes this very likely.

One possibility would be that this type of recombination is common to all antigen receptor genes and has not been identified previously. In this scenario, rearrangements distal to the JC locus might be a necessary step in opening up the chromosome for further rearrangement and expression. This might be brought about by either the deletion of a negative regulatory element in this region or the introduction of a positive one. A second possibility is that this is necessary for antigen receptor genes that appear late in lymphocyte differentiation and that the purpose may be to provide a high-affinity site for recombinase distal to the JC locus, which in some way prevents premature VJ rearrangement and expression. A third possibility is that the 5' C_α locus involves some other rearranging gene. After this manuscript was submitted, we found that this is in

fact the case, and that a new TCR gene, termed T cell receptor X is encoded in this region, and the rearrangements that we see involve J and C region elements distinct from those encoding TCR α polypeptides (26). The early onset of rearrangement and expression of this new TCR gene make it a possible candidate for the TCR δ chain polypeptide (26).

Summary

The dull Ly-1 double-negative (Ly-1^{dull}, Lyt-2⁻, L3T4⁻) subpopulation appears to be the major precursor group of T lymphocytes in the thymus. In examining the status of the α , β , and γ chain genes for T cell receptors (TCR) in this population of cells and hybridomas made from them, we find that all of these loci appear to begin DNA rearrangements in a nearly simultaneous fashion. In the case of the γ genes, these involve $V_\gamma \rightarrow J_\gamma C_\gamma$ gene rearrangements; with the β chain genes, both $D_\beta \rightarrow J_\beta C_\beta$ rearrangement and $V_\beta \rightarrow D_\beta J_\beta C_\beta$ rearrangements are evident; and in the case of the α locus, assayed in part by pulsed-field gel electrophoresis, they take the form of a novel series of rearrangements occurring 80 kb or more 5' to the C_α gene. These α locus rearrangements are well away from any of the J_α gene segments found in cDNA clones to date and are deleted in most mature thymocytes and functional T cell lines. Therefore they appear to represent a distinct class of rearrangement that occurs before $V_\alpha \rightarrow J_\alpha$ joining. These distinctions between the character of the TCR gene rearrangements in these cells represent useful markers in further distinguishing different stages of T cell differentiation within this compartment of early T cells. In addition, the unexpected discovery of clonal rearrangements so far away from any of the expressed J_α gene segments, and at a stage where there is little or no stable C_α RNA present, has interesting implications for the hierarchy of TCR gene expression.

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