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Variable region (V_δ) gene segment most frequently utilized in adult thymocytes is 3′ of the constant (C_δ) region

(T-cell development/DNA rearrangement/γ–δ heterodimer/T-cell receptor)

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ABSTRACT **A variable region ($V_\delta5$) of the T-cell receptor (TcR) δ chain that is preferentially expressed in adult murine thymocytes is located 2.5 kilobases 3′ of the constant region (C_δ) element. The $V_\delta5$ coding sequence is in a transcriptional orientation opposite the J_δ (joining region) and C_δ coding elements and rearranges by inversion. The C_δ is divided into four exons, three of which encode amino acids of TcR δ polypeptide, and the fourth comprises the entire 3′ untranslated region. In this respect, C_δ resembles C_α rather than C_β or C_γ.**

Two major types of T-cell receptor (TcR) heterodimer, α–β (1–3) and γ–δ (4–9), have been observed on the surfaces of T cells, each in association with the CD3 polypeptides. Whereas the TcR α–β-bearing cells encompass all known helper and cytotoxic T cells, the function(s) of TcR γ–δ cells is not yet clear. During thymic ontogeny, γ–δ T cells appear before those bearing α–β (6, 7). In the adult thymus, γ–δ-bearing T cells are present in the least mature $CD3^+4^-8^-$ double-negative (DN) (10) population of T cells. γ–δ T cells can also be found in skin dendritic cells (11) and in a small percentage of peripheral T cells (4). We have recently identified the gene segments that encode variable (V), diversity (D), joining (J), and constant (C) regions of the TcR δ chain and have shown that they are largely contained within the TcR α locus (12–14).

V_δ rearrangements involving $V_\delta1$ (V_{M21} in ref. 13) predominate in T-cell hybridomas made from early fetal thymocytes (days 14–16 of gestation). $V_\delta1$ also accounts for the majority of VDJ_δ rearrangements identified in a day 18–19 total thymocyte genomic library (13). mRNAs containing $V_\delta1$ sequences are readily detectable in fetal thymocytes (data not shown). However, no $V_\delta1$ sequences are present in 21 cDNA clones containing V-region sequences isolated from adult DN thymocyte cDNA libraries. Instead, another V_δ sequence ($V_\delta5$/Z44) accounted for 11 of the 21 clones (14). The other V_δ sequences used by adult DN thymocytes also differ substantially from V_α and fetal V_δ sequences. In this report, we show that the abundance of $V_\delta5$ transcripts in the adult DN cell population reflect a switch in the control of V_δ rearrangements between fetal and adult populations. We also show that $V_\delta5$ is located 3′ of the C_δ coding region and rearranges by inversion. A V gene segment located 3′ of a C gene segment has previously been identified in the TcR β locus. However, the organization of the C_δ exons is similar to TcR α and unlike that of the β and γ loci.

MATERIALS AND METHODS

High molecular weight DNAs were isolated, digested with restriction enzymes, electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose filters. All the blots were hybridized in 1 M NaCl/50 mM $NaPO_4$, pH 6.5/50% formamide/salmon sperm DNA (100 μg/ml)/5× Denhardt's solution (1× Denhardt's solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone)/10% dextran sulfate at 40°C with random hexamer labeled probes (15) and washed as described (13). To determine the genomic organization of C_δ and $V_\delta5$, a cosmid clone COS 3 derived from a library made from embryonic DNA of the BALB/c strain (16) was analyzed with radiolabeled probes from C_δ and $V_\delta5$-containing cDNA clones (pλ12 and Z44, respectively) (12, 14). For DNA sequencing, all the DNA fragments were subcloned into the PGEM-3 vector and the sequences were determined by the dideoxy nucleotide chain-termination method with both SP6 and T7 promoter-specific primers on double-stranded plasmid DNA using the large fragment of DNA polymerase I (Boehringer Mannheim) or the modified T7 polymerase (Sequenase, United States Biochemical, Cleveland) (17). To facilitate sequencing, a DNA oligomer (17 nucleotides) specific to part of the first exon of the C_δ gene was synthesized and used as a primer. In addition, deletional subclones were generated by *Exo* III and mung bean nuclease treatment (18).

RESULTS

V_δ Rearrangements in Fetal and Adult Thymocytes. To better understand the switch in expression between the two V_δ gene segments that predominate in either the fetal or adult thymocyte populations ($V_\delta1$ or $V_\delta5$, respectively), we performed Southern blot analysis with these probes to compare rearrangements in total fetal thymocyte cells and adult DN cells. As shown in Fig. 1, $V_\delta5$ rearrangements can be detected in adult DN thymocytes and in total adult thymocyte DNA but not in fetal cells. In contrast, $V_\delta1$ shows rearrangements in day 16 and day 19 fetal thymocytes but not in the adult population. Similar results were also obtained when a γ–δ-bearing subset of adult DN cells was used instead of the total DN population (D. M. Pardoll, personal communication). This indicates that the abundance of $V_\delta5$ transcripts in adult DN cell populations is not the result of overexpression of $V_\delta5$-containing mRNA by a few cells or underexpression of $V_\delta1$ transcripts by large numbers of adult thymocytes but instead reflects a switch in the control of V_δ rearrangements between the two populations. Southern analysis shows that the change in specificity of rearrangement between fetal and adult cell populations extends to the D_δ and J_δ elements. Bands corresponding to $V_\delta1$ rearranged to $D_\delta2$, $(D)J_\delta1$, and $(D)J_\delta2$ are all evident in fetal cells, but in the adult thymocyte population, only a single rearranged band is detected by $V_\delta5$ and this corresponds to a $V(D)J_\delta1$ joining.

Location of $V_\delta5$ and the Sequence Organization of $V_\delta5$ and C_δ. In the course of analyzing the structural basis for the specificity of $V_\delta1$ and $V_\delta5$ gene rearrangement, we isolated

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Abbreviations: V, variable; D, diversity; J, joining; C, constant; DN, double negative; TcR, T-cell receptor.

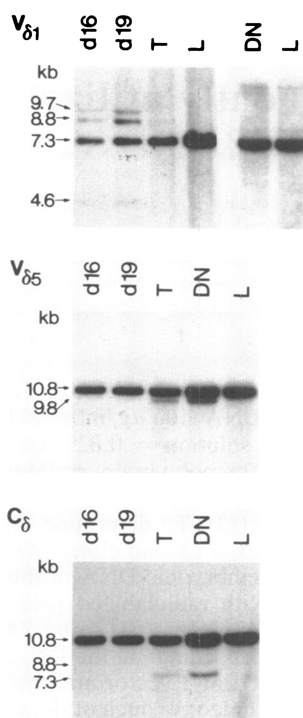

FIG. 1. Southern blot analysis of rearrangement in fetal and adult thymocytes as detected by $V_\delta1$, $V_\delta5$, and C_δ probes. Aliquots of 8 μg of *Eco*RI-digested genomic DNA from day 16 (d16) fetal thymocyte, day 19 (d19) fetal thymocyte, adult $CD4^-8^-$ thymocyte (DN), adult total thymocyte (T), and liver (L) DNA were electrophoresed on a 0.8% agarose gel, blotted onto nitrocellulose, and hybridized with a $V_\delta1$ probe (*Upper*) [described previously as the V_{M21} probe (13)]; a 0.8-kb fragment containing mostly 5′ flanking region of V_{M21}. It detects a 7.3-kb germ-line band, a 9.7-kb band in a $V_\delta1D_\delta2$ rearrangement, and 8.8- and 4.6-kb bands corresponding to $V_\delta1(D)J_\delta1$ and $V_\delta1(D)J_\delta2$ types of rearrangement, respectively. (*Middle*) $V_\delta5$ probe: a 390-base-pair *Eco*RI/*Ava* II V-region-specific probe from Z44 containing plasmid (14). It detects a 10.8-kb germ-line band and a 9.8-kb band corresponding to a $V_\delta5(D)J_\delta1$ rearrangement. (*Lower*) C_δ probe: a 0.9-kb *Eco*RI fragment from a pλ12 TcR δ-chain clone containing the 3′ portion of the C region (12). It detects a 10.8-kb germ-line band, a 7.3-kb rearranged band corresponding to the reciprocal joining product of the region 3′ of $V_\delta5$ and the $D_\delta1$ locus, and an 8.8-kb rearranged band corresponding to the reciprocal joining product of the region 3′ of $V_\delta5$ to the $D_\delta2J_\delta1$ locus.

$V_\delta1$ and $V_\delta5$-containing clones from a mouse (B10.A) liver genomic library (19). Analysis of the $V_\delta5$-containing clones revealed that $V_\delta5$ is located on the 3′ side of the C_δ coding region. The subsequent analysis of the $V_\delta5$ gene was done on subclones containing the C_δ locus that we had previously isolated (12) from a BALB/c germ-line cosmid library (16). Fig. 2 shows the linkage between the C_δ and $V_\delta5$ regions with a restriction endonuclease map and Fig. 3 indicates the sequences of the four C_δ exons and the two $V_\delta5$ exons. This analysis shows that $V_\delta5$ is in a transcriptional orientation opposite that of $J_\delta C_\delta$. This is similar to the TcR β locus where a single V_β element, $V_\beta14$, is located 10 kilobases (kb) 3′ of $C_\beta2$ and is in a transcriptional orientation opposite that of $J_\beta C_\beta$ coding sequences (23). Despite this similarity in location, the usage of these two V regions differs greatly. $V_\delta5$ is the most frequently used V_δ region in adult thymocytes, but $V_\beta14$ is among the least frequently used of the V_β gene segments (1–2%) in adult T cells (24).

The intron–exon organization of C_δ is very similar to C_α and less related to C_β and C_γ. The first exon encodes the single immunoglobulin-like domain characteristic of all TcR genes and contains the two cysteines, which have been suggested as candidates for an intramolecular disulfide bond.

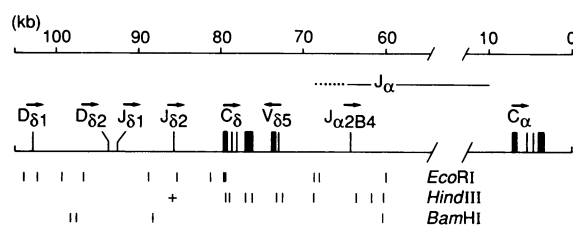

FIG. 2. Genomic organization of TcR δ locus. A restriction map of the δ locus and its location with respect to the J_α and C_α coding regions. The identification of $D_\delta1$, $D_\delta2$, $J_\delta1$, $J_\delta2$, and $J_\alpha2B4$ has been described (12, 13). The positions of C_δ and $V_\delta5$ exons were determined by restriction enzyme mapping, Southern analysis, and DNA sequencing.

Although we found a very low (≈15%) degree of amino acid similarity to constant regions of immunoglobulin μ and $\lambda1$ chains (12), A. Williams (personal communication) has found that the murine C_δ sequence shows a 32% amino acid similarity to human immunoglobulin $C_\gamma1$ in this region. This reinforces the supposition that this exon would form an immunoglobulin-like domain.

The second exon encodes the largest of the TcR hinge-like regions and is relatively rich in proline residues (4 prolines in 21 amino acids). The number of prolines in this region in the TcR δ chain makes it similar to immunoglobulin heavy-chain hinge regions and different from other TcR polypeptides. The α chain contains only one proline in the second exon. Although the β chain has a very short second exon with no proline residues, the C-terminal end of the first exon of the β chain encodes a cluster of three prolines. This cluster might form a different structure from that of the δ chain because of its distance from the conserved cysteine in the hinge region. The mouse γ chain has no proline encoded in the second exon and there is no cluster of prolines in the C region.

The third exon contains the transmembrane and cytoplasmic regions. Similar to other TcR molecules, the transmembrane region is hydrophobic except for a highly conserved lysine residue (Fig. 3*i*, solid arrowhead). The positively charged lysine residue may be involved in an interaction with negatively charged residues in the CD3 polypeptides (22). In addition, α, β, and δ chains have another charged amino acid. This amino acid is always the same distance from the conserved lysine (Fig. 3*i*, open arrowhead). The α and δ chains have a positively charged arginine residue at this position, and the β chain has a negatively charged aspartic acid. The γ chain, however, has a nonpolar leucine residue at this position. This suggests that γ–δ C regions may have different heterodimeric structures from α–β and may therefore interact differently with the CD3 complex.

The fourth exon encodes the 3′ untranslated region. The organization is similar to that of the TcR α-chain C region but different from TcR β and γ genes as well as immunoglobulin genes in which the terminal exons contain a short coding region in addition to the 3′ untranslated regions (22, 25).

When the genomic sequence of $V_\delta5$ was compared with the published cDNA sequences (14), three nucleotide differences were observed; however, none of these differences cause a change in amino acid sequence in the V region of the mature protein (Fig. 3*ii*). It is not clear whether these differences arise from a polymerase error during generation of the cDNA clones, strain polymorphism [the cDNA clones were from (BALB/c × 129)F_1 RNA-derived libraries, and the genomic sequence is from a BALB/c-derived cosmid library], or somatic mutations. This sequence comparison also shows that the leader sequences reported by Elliott *et al.* (14) for three $V_\delta5$-containing cDNA clones are in fact contiguous with the genomic $V_\delta5$ sequence reported here. This suggests that these cDNA clones were derived from unspliced mRNA.

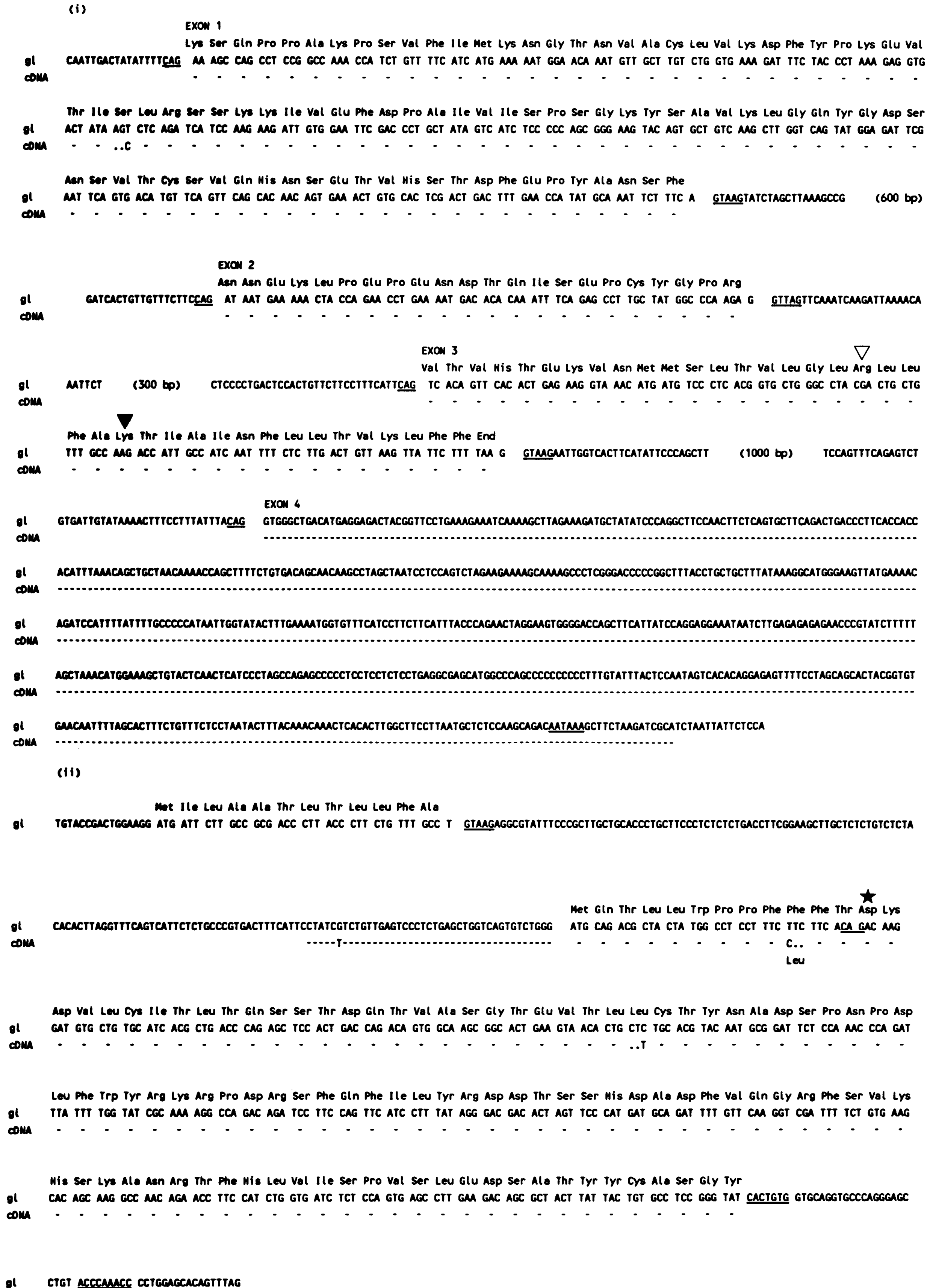

FIG. 3. Sequences of the C_δ and $V_\delta5$ exons. Germ-line (gl) nucleotide sequences of the (*i*) four C_δ and (*ii*) two $V_\delta5$ exons were determined by sequence comparison with the cDNA clones pλ12 for C_δ (12) and Z72 for $V_\delta5$ (14). Consensus splicing signal sequences (20), polyadenylylation signals (21), and heptamer nonamer sequences (22) are underlined. When the genomic sequence differs from that of the cDNA clone, the nucleotide from the cDNA clone is indicated below the genomic sequence. ★, Amino acid can be either an Asp (GAC) or a Tyr (TAC) due to splicing from the putative leader sequence. Arrowheads indicate charged amino acids in the transmembrane region. bp, Base pairs.

In fact, a "leader-like" sequence can be found 196 base pairs 5′ of the V region (as indicated in Fig. 3*ii*) and is flanked by a proper splicing consensus sequence. The sequence encodes the same number of amino acids as the previously reported sequence. They are, however, a somewhat more hydrophobic stretch of amino acids and thus may be the real leader sequence. Alternatively, both may be used as the leader peptide.

$V_\delta5$ Is Rearranged by Inversion. Because $V_\delta5$ is in an opposite transcriptional orientation relative to the $D_\delta J_\delta C_\delta$ sequences, it must rearrange by inversion to be expressed. The reciprocal joint product should be retained in the genome and the sequences in between the joined gene segments should be moved to a new location. That $V_\delta5$ is indeed rearranged by inversion is evident in the Southern blot analysis of two hybridomas (1D5 and 2B6) made from the $CD5^{low}$ subset of adult DN thymocytes (26) (Fig. 4*A*). As schematically diagrammed in Fig. 4*B* in 1D5, $V_\delta5$ has undergone a $V_\delta5D_\delta1(D_\delta2)J_\delta1$ rearrangement. The region between $D_\delta1$ and $J_\delta1$ has been deleted. This conclusion is supported by further Southern analysis, which shows that (*i*) both a C_δ probe and a 1.9-kb $D_\delta1$ probe hybridize to the same rearranged 7.3-kb band, (*ii*) $V_\delta5$ and a probe containing sequence 3′ of $J_\delta1$ (data not shown) hybridize to the same 9.8-kb band, and (*iii*) that sequences between $D_\delta1$ and $J_\delta1$ (the DJ probe) and a 5′ $D_\delta1$ probe fail to show any hybridization (data not shown). Whether $D_\delta2$ is involved in this joining is not clear from the Southern analysis. This reciprocal joining product can be generated either by $V_\delta5$ $D_\delta1$ rearrangement with subsequent $D_\delta1(D_\delta2)J_\delta1$ joining (13) or by $V_\delta5$ rearrangement to a preexisting $D_\delta1(D_\delta2)J_\delta1$ joint. In the hybridoma 2B6, $V_\delta5$ hybridizes to a rearranged 9.8-kb band, which also hybridizes to the 3′ $J_\delta1$ probe (data not shown), but C_δ, 5′ D, and DJ probes hybridize to another rearranged band of 8.8 kb. This

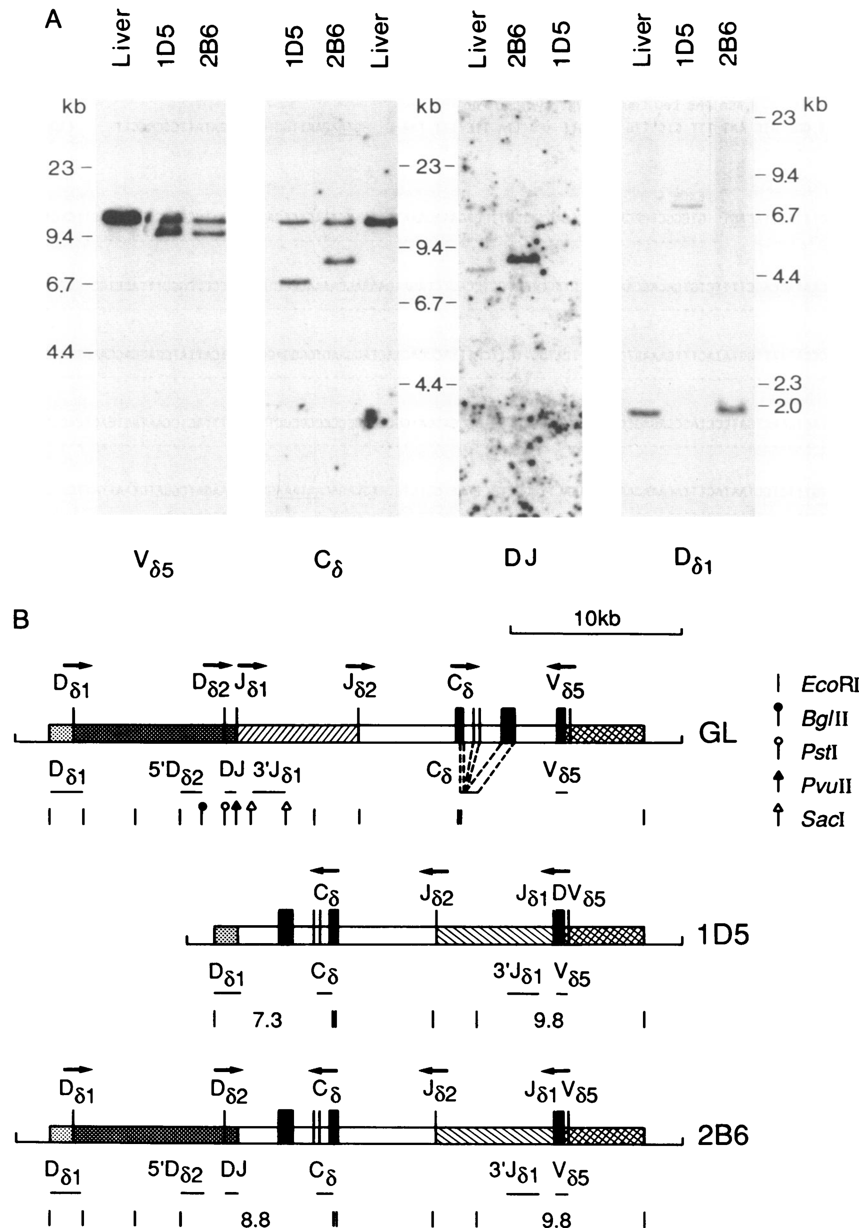

FIG. 4. Southern blot analysis of 1D5 and 2B6 DNA and a schematic representation, containing the location of probes, of the restriction map of the germ-line (GL) and rearranged chromosomes in 1D5 and 2B6. (*A*) Aliquots of 8 μg of *Eco*RI-digested DNA from liver (L) hybridomas 1D5 and 2B6 were electrophoresed in 0.8% agarose, blotted onto nitrocellulose, and hybridized with the $V_\delta5$, C_δ, DJ, and $D_\delta1$ probes as described in Fig. 1. (*B*) The locations of D, J, C, and V gene segments and the probes have been described (Fig. 1; ref. 13). The transcriptional orientation of each gene segment is indicated above the element.

suggests that in this cell line $V_\delta 5$ has rearranged to $J_\delta 1$ and the joining involves neither $D_\delta 1$ nor $D_\delta 2$. That $D_\delta 1$ is not involved in this rearrangement is further confirmed by the fact that the $D_\delta 1$ probe hybridizes to the same 1.9-kb *Eco*RI fragment as germ-line DNA. The direct joining of a V to J without the participation of a D element has been seen (albeit rarely) in the TcR β locus (27) and is compatible with the arrangement of heptamer and nonamer sequences around the TcR δ gene segments (13).

Further confirmation that $V_\delta 5$ can undergo VDJ_δ rearrangements that involve either the $D_\delta 1$ locus or the $D_\delta 2 J_\delta 1$ locus is the fact that in adult cells only a single rearranged band can be detected with a $V_\delta 5$ probe but that two major rearranged bands (7.3 and 8.8 kb) can be detected when C_δ is used as a probe (Fig. 1). Southern analysis with the $D_\delta 1$ probe confirmed that the 7.3-kb band corresponds to joining (by inversion) between the 3′ end of $V_\delta 5$ and the $D_\delta 1$ locus. The 8.8-kb band corresponds to the 3′ flanking region of $V_\delta 5$ joining at the $D_\delta 2 J_\delta 1$ locus. That the 8.8-kb band is far less intense than the 7.3-kb band suggests that most or all of the $V_\delta 5$ rearrangements involve $D_\delta 1$. This result is consistent with the finding that most of the cDNA clones isolated from an adult DN thymocyte library show a $D_\delta 1$ contribution in their assembled V regions (14).

DISCUSSION

The data presented here add to our earlier findings (13, 14) that utilization of different gene segments at the δ locus is tightly regulated in different T-cell populations. In fetal thymocytes, one particular V region ($V_\delta 1$) is preferentially rearranged, and most of the rearrangements involve $D_\delta 2$ rather than $D_\delta 1$. In adult cells, a different set of V_δ elements is used, and most of the functional rearrangements involve both $D_\delta 1$ and $D_\delta 2$.

As major changes and rapid turnover in T-cell subpopulations have been reported during thymic development (28), this observation could reflect a selective mechanism depending on the surface expression of γ–δ receptor. It is also possible that different gene segments are preferentially "activated" for rearrangement in different cell types. $V_\delta 1$ is the first V_δ gene segment that rearranges at the δ locus during the ontogeny of fetal thymocytes. In fetal thymic cell populations and hybridomas, it is frequently found in aberrant VD joints and in out-of-frame VDJ rearrangements. Since the latter is a nonfunctional rearrangement, cells with this type of rearrangement could not expand because of preferential selection against specific γ–δ heterodimers. Therefore, the high frequency of rearrangement of $V_\delta 1$ observed in fetal thymocytes is most likely due to an intrinsically high rate of rearrangement in fetal thymocytes. Although we have not linked $V_\delta 1$ to the $D_\delta J_\delta C_\delta$ coding regions, it must be at least 40 kb 5′ of $D_\delta 2$ (data not shown), a distance much greater than that between $V_\delta 5$ and $D_\delta 2$ (20 kb). These data, together with the observation that the $VD_\delta 2$ and $D_\delta 1 D_\delta 2$ types of rearrangement are more frequently observed in fetal thymocytes than $D_\delta 2 J_\delta 1$ joining despite the close proximity of $D_\delta 2$ and $J_\delta 1$ (0.9 kb) (13), suggest that factors other than simple proximity are playing an important role in both the process of rearrangement and the regulation of V region usage in different populations of T cells.

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