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Sequence and organization of the diversity, joining, and constant region genes of the human T-cell δ-chain locus

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ABSTRACT In this paper we describe the genomic organization and sequence of the human T-cell receptor δ-chain diversity, joining, and constant genes. There is one δ-chain constant region gene (C_δ) located ≈85 kilobases (kb) upstream of the α-chain constant region. The δ-chain constant region consists of four exons, whose organization is very similar to that of the C_α exons, suggesting that C_α and C_δ may have arisen from a gene duplication event. The first exon encodes most of the extracellular constant domain, the second encodes a hinge-like region, and the third encodes the entire transmembrane segment and intracytoplasmic portion, whereas the last exon contains exclusively 3′ untranslated sequences. Three joining segments, $J_\delta1$, $J_\delta2$, and $J_\delta3$, are found ≈12, ≈5.7, and ≈3.4 kb upstream of the first exon of C_δ. Two functional diversity gene segments, $D_\delta1$ and $D_\delta2$, which can be productively translated in all three reading frames, are found 1 and 9.6 kb upstream of $J_\delta1$. The presence of two D_δ with such potential for diversity may offset the limited repertoire of the J_δ and V_δ genes. The spacer distribution in the recombinational signals flanking D_δ and J_δ segments allows recombination with V_α gene segments; however, examination of δ-chain messages does not indicate that this is the case, suggesting that the δ chain uses unique variable gene segments and raising the question as to the reasons for this phenomenon.

The genes of the α and β chains of the T-cell antigen receptors consist of noncontiguous variable (V), diversity (D), joining (J), and constant (C) gene segments (1–6), which undergo somatic rearrangement in T cells during ontogeny to form the functional α- and β-chain genes (6–12). The α- and β-chain loci are composed of a large number of V, D, and J gene segments, endowing these two chains with a high degree of combinational diversity (13).

In the course of the search for the α-chain genes, a third T-cell-specific rearranging locus, the γ-chain locus, was found (14). Like the α- and β-chain loci, the γ locus consists of noncontiguous V, J, and C gene segments (15–18). In most cases, the γ gene products are associated on the cell surface with the recently identified δ-chain protein (19–27). The δ-chain C region is nested in the α-chain locus, upstream of the C_α and J_α genes (27). In this study, we have determined the genomic organization of the δ-chain gene locus in humans. The nucleotide sequence and organization of the C region gene and J gene segments and their locations are provided.* In addition, we found that the δ-chain locus contains two D gene segments.

MATERIALS AND METHODS

Genomic Clones. Human genomic clones were isolated from the Maniatis genomic library (28). Over a dozen overlapping clones were obtained by using the δ-chain cDNA described by Takihara *et al.* (27) as a probe. The final genomic map was constructed by combining the restriction maps of these overlapping clones and using the technique of partial digestion (29).

DNA Sequencing. Sequencing of the genomic DNA was performed using a combination of shotgun sequencing and the specific primer-directed dideoxynucleotide sequencing technique. After fine genomic mapping of the regions of interest, DNA fragments were subcloned in M13mp8 or M13mp9 and their sequences were determined by using the Sanger dideoxy method (30). When necessary, the specific primer-directed dideoxynucleotide sequencing method was performed using oligonucleotides.

RESULTS

Genomic Organization of the Human δ-Chain Gene. A restriction enzyme map of the 40 kilobases (kb) of DNA that encompasses the human δ germ-line region is shown in Fig. 1. As can be seen, the C region gene is located ≈85 kb upstream of the C_α region (31). The restriction map in Fig. 1 and the sequences of the intron–exon junction and splice donor sites illustrated in Fig. 2 show that the C_δ gene is composed of four exons that span ≈4 kb. Comparison of the genomic organization with the deduced protein structure of the C region gene reveals that the first exon encodes the majority of the extracellular domain (from amino acids 1 to 92 of the C region). The second exon codes for a hinge-like region and the third exon consists mainly of the transmembrane and intracytoplasmic sequences, whereas the last exon is made up of only 3′ untranslated sequences. There are two poly(A) addition signals 700 base pairs (bp) apart (underlined in Fig. 2), either of which can be used. Thus the four sizes of δ-chain messages consist of two functional messages of 1.5 and 2.2 kb, each using a different poly(A) site, and two nonfunctional JC transcripts of 2 and 1.3 kb. The three nucleotides of our sequence that differ from previously published cDNA sequences (22, 24, 27) are indicated in Fig. 2. We have previously noted that the deduced protein sequence of the C_δ gene is highly homologous to that of C_α, suggesting a close evolutionary relationship between these two genes. On examination of the genomic organization of the exons and introns of the C_δ genes, it is clear that the organization of this newly identified T-cell rearranging gene is also very similar to that of C_α (8), lending support to the hypothesis that the C_δ and C_α genes arose by a gene duplication event.

There Are Three J_δ Gene Segments. By using oligonucleotide probes that correspond to J gene sequences, we have located three regions containing J gene sequences located ≈3.4, ≈5.7,

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Abbreviations: C, constant; D, diversity; J, joining; V, variable.
*The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03837).

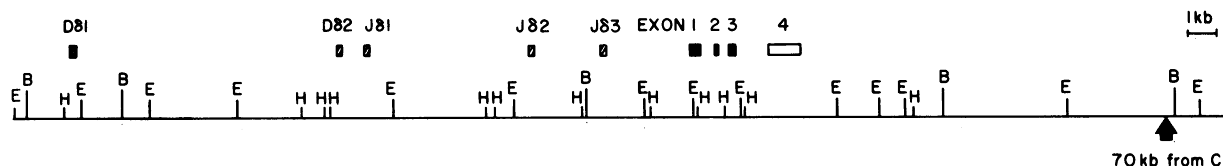

FIG. 1. Restriction enzyme map of the human T-cell receptor δ-chain locus. The map was deduced by analysis of the recombinant clones that hybridized with the δ-chain cDNA described previously (27), using the restriction enzymes *Eco*RI (E), *Bam*HI (B), and *Hin*dIII (H). Open and closed boxes indicate 3′ untranslated region and coding exons, respectively. Striated boxes show the J_δ and D_δ regions.

and ≈12 kb upstream of the first exon of the C_δ gene (Fig. 1). Examination of sequences surrounding these two regions (see Fig. 3) does not reveal any other J-like sequences. The three J_δ gene segments contain the consensus J gene sequence of Phe-Gly-Xaa-Gly found in almost all other immunoglobulin and T-cell receptor J gene segments. In addition, recombination signals consisting of the heptamers and nonamers separated by 12- or 13-nucleotide nonconserved spacer sequences can be found 5′ to each of the J gene segments. $J_\delta1$, $J_\delta2$, and $J_\delta3$ contain 16, 17, and 19 amino acids, respectively. Proper splice signals are located 3′ of each of these J gene segments. Comparison of the human and murine germ-line J_δ sequences shows that the nucleotide and amino acid sequences are highly conserved between human and murine $J_\delta1$ (Fig. 3). Furthermore, the nucleotide and amino acid sequences of human $J_\delta3$ are significantly homologous to those of murine $J_\delta2$, suggesting that these J_δ sequences may play an important role in T-cell receptor δ-chain function. Alignment of the germ-line sequences with the cDNA sequences is illustrated in Fig. 4 *Lower*. The finding of three functional J genes using J-specific probes suggests that three J gene segments are associated with δ-chain genes. However, the possibility remains that there are additional J_δ that have little or no homology to our J oligonucleotide probe.

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            ............................ JC INTRON ...............................

            .............CACTAACAGGATGCATGAGGTCTGGATTGTAGTGTTTGGCTCCAGGGTAATCGAGGT

            AATCACCACTGTTTAACCCCCACAAAGTTGTGAATAATCATCTCACCTAATAAGTTGATTATATTTGCAG

              SerGlnProHisThrLysProSerValPheValMETLysAsnGlyThrAsnValAlaCysLeuValLys
            GAAGTCAGCCTCATACCAAACCATCCGTTTTTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAA

             GluPheTyrProLysAspIleArgIleAsnLeuValSerSerLysLysIleThrGluPheAspProAla
            GGAATTCTACCCCAAGGATATAAGAATAAATCTCGTGTCATCCAAGAAGATAACAGAGTTTGATCCTGCT
EXON 1
            IleValIleSerProSerGlyLysTyrAsnAlaValLysLeuGlyLysTyrGluAspSerAsnSerVal
            ATTGTCATCTCTCCCAGTGGGAAGTACAATGCTGTCAAGCTTGGTAAATATGAAGATTCAAATTCAGTGA

           ThrCysSerValGlnHisAspAsnLysThrValHisSerThrAspPheGluValLysThrAspSerThr
            CATGTTCAGTTCAACACGACAATAAAACTGTGCACTCCACTGACTTTGAAGTGAAGACAGATTCTACAGG

            TAGGCCATTTCTAGCTTCAAGG ..... FIRST INTRON ........ GACCACTGCTGTTTGTTCC

             AspHisValLysProLysGluThrGluAsnThrLysGlnProSerLysSerCysHisLysProLys
EXON 2      AGATCACGTAAAACCAAAGGAAACTGAAAACACAAAGCAACCTTCAAAGAGCTGCCATAAACCCAAAGGT

            TAGTTCAAATCAAAGGGCCAAG ..... SECOND INTRON ....... ACCCTCTTCACTCTTTTTC

             AlaIleValHisThrGluLysValAsnMETMETSerLeuThrValLeuGlyLeuArgMETLeuPheAla
            AGCCATAGTTCATACCGAGAAGGTGAACATGATGTCCCTCACAGTGCTTGGGCTACGAATGCTGTTTGCA
EXON 3
            LysThrValAlaValAsnPheLeuLeuThrAlaLysLeuPhePheLeu***
            AAGACTGTTGCCGTCAATTTTCTCTTGACTGCCAAGTTATTTTTCTTGTAAGGTAAGAATTAGCCGCTTC

            TTATT ...................... THIRD INTRON ......... AACAACTTTCTTCACTGC

            AGGCTGACTGGCATGAGGAAGCTACACTCCTGAAGAAACCAAAGGCTTACAAAAATGCATCTCCTTGGCT
                                                                          ●1     ●2
            TCTGACTTCTTTGTGATTCAAGTTGACCTGTCATAGCCTTGTTAAAATGGCTGCTAGCCAAACCAATTTT

            TCTTCAAAGACAACAAACCCAGCTCATCCTCCAGCTTGATGGGAAGACAAAAGTCCTGGGGAAGGGGGGT

            TTATGTCCTAACTGCTTTGTATGCTGTTTTATAAAGGGATAGAAGGATATAAAAAGATATAGGACTCTTT

            TTTTACTCCTACAAGTGATACACTTTGAAAATGATGTTTTGTTCCTTTTGACTTTCTTTACCTTTTGAAG

            TAGAAAGTGGGAACCAACAGGTTCACAGCTTCATTCCTCATGAGGAAAATAGGCCTTGGGAGAAGAAGAG

            CGGGTGCCCTTTTATCTAAACATGGAAGGCTCTGCTCAACTGAGCACTAGATTTGCTACAAACCAGCATC
EXON 4
            ATCTTCTTCCTCCTGTCCTCACGGCTTGTCCCACCCTCTATGTTCACTTCAGGAGCCACACTAGAGATTC

            TGCATGGCGTGGAGGACAAAGTTTCAGCACTTTCTGCCTCTCCTAATACTTTACAAATGAGATTACATTT

            GAATTTGCTAATACTTTATGAGCAGGCAATGAGGTTTCCAAAATCTCATCTAAATACTCTCCAATCTATT

            AGCAAAAATCAGAGTAAAATACAGAGGAAAGGCACTGCTTTCTGTTAATTGATTTAACATGCATGAATTA

            GCTCCCTCTGAGTTCCAGGCACTATGCTGAGAGTACAAAGAAGACACAAGTCTGCTTTCAAGCAACTCAC

            TGTGAAAGTGTTTTTGAAGGGAGGAACAGAAATGAGACCCCTATCTTTCCCTATAAAAACAACATTTTTA
                                                                                 ●3
            CTGTGTTTTGCCTGCCAATCTGTATTTGAAACCATTGGACACTGATTCTCTGGCCTGGGACTTTGGCATT

            GATGGTTTTCTGCCTTTCTTCTCAGCCTCTGCCTCTATTGCATTTATTAAACTGCATTGTGTGCACCTCG

            CCTCTGGCTTTACTCTTTGCAGATCACCACAGGGGGAAACTCAGCTCTGTGAGCTCACTATTAGT
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FIG. 2. Partial nucleotide sequence of the human genomic C_δ region. Conserved splice signals are indicated by dotted lines, and poly(A) signals are underlined. Exons are boxed with amino acid sequences. The nucleotides that differ from cDNA sequences reported previously (22, 24, 27) are marked with closed circles.

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Human                                           ThrAspLysLeuIlePheGlyLys
   Jδ1   GCGCTGAGGTTTTTGGAACGT-CCTCAAGTGCTGTGACACCGATAAACTCATCTTTGGAAAA
Mouse      **************** *  *****   ******  ***** ****** ******** **
   Jδ1   CTGCTGAGGTTTTTGGAATGGGCCTCAGTAGCTGTGCTACCGACAAACTCGTCTTTGGACAA
                                                ThrAspLysLeuValPheGlyGln

Human    GlyThrArgValThrValGluPro
   Jδ1   GGAACCCGTGTGACTGTGGAACCAAGTAAGTAACTCATT
Mouse    *******  ********************** * * **
   Jδ1   GGAACCCAAGTGACTGTGGAACCAAGTAAGTCATTTATC
         GlyThrGlnValThrValGluPro

Human                                           ThrAspLysLeuIlePheGlyLys
   Jδ1   CTGAGGTTTTTGG-AACGTCCTCAAGTGCTGTG---ACACCGATAAACTCATCTTTGGAAAA
Human       ******** * ** *    *    *  ***     ** *   ***** **********
   Jδ2   GCAAGGTTTTTCGTAATGATGCCTGTGGTAGTGCTTTGACAGCACAACTCTTCTTTGGAAAG
                                            LeuThrAlaGlnLeuPhePheGlyLys

Human    GlyThrArgValThrValGluPro
   Jδ1   GGAACCCGTGTGACTGTGGAACCAAGTAAGTAACTCATTTATTTATCTGAAGTTTAAGGTTA
Human    ***** *   * *  ********** ******* *  *****   **           *
   Jδ2   GGAACACAACTCATCGTGGAACCAGGTAAGTTATGCATTTTACTACAGCTTCAGGGGAAAC
         GlyThrGlnLeuIleValGluPro

Human                                       SerTrpAspThrArgGlnMetPhePhe
   Jδ3   AGGCTAGTTACCTGTGAGGCACTGTCATAATGTGCTCCTGGGACACCCGACAGATGTTTTTC
Mouse    ** ** **** ***  * ***   * **** ******************************
   Jδ2   AGACTGGTTATCTGCAAAGCAAGATTATAACGTGCTCCTGGGACACCCGACAGATGTTTTTT
                                            SerTrpAspThrArgGlnMetPhePhe

Human    GlyThrGlyIleLysLeuPheValGluPro
   Jδ3   GGAACTGGCATCAAACTCTTCGTGGAGCCCCGTGAGTTGATCTTTTTCC
Mouse    ***********  * ***** *********** ****** * ***** *
   Jδ2   GGAACTGGCATAGAGCTCTTTGTGGAGCCCCGTAAGTTGGTTTTTTTTC
         GlyThrGlyIleGluLeuPheValGluPro
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FIG. 3. Comparison of human and murine germ-line J_δ sequences. Spaces have been inserted to maximize homology among the different J_δ sequences. Homology between the sequences is indicated by asterisks. The conserved nonamer and heptamer sequences are boxed.

Human δ-Chain Sequences Contain Two D Gene Segments. By using oligonucleotide probes homologous to D gene sequences derived from cDNA sequences and probes homologous to consensus heptamer and nonamer under nonstringent hybridization conditions, we found two D_δ gene segments within the 50-kb region upstream of the C_δ. $D_\delta 1$ and $D_\delta 2$ have all of the properties of functional D regions and are located ≈1 and ≈9.6 kb 5′ to $J_\delta 1$. Fig. 4 *Upper* shows the 1348-nucleotide sequence and the restriction enzyme map of the region surrounding $D_\delta 2$. $D_\delta 1$ and $D_\delta 2$ genes can be read in all three possible translational reading frames to produce sequences two to four amino acid residues in length and are flanked on both sides with heptamers and nonamer sequences separated by spacers. The 5′ spacer is 12 nucleotides, whereas the 3′ spacer is 23 nucleotides long. Both D_δ sequences are present in the group O cDNA clone reported by Hata *et al.* (22), strongly suggesting that this is a functional D gene segment (Fig. 4 *Lower*). The presence of two such flexible D regions greatly increases the potential diversity of the δ chain.

DISCUSSION

We describe here the nucleotide sequence and genomic organization of the D, J, and C region genes of the human T-cell δ-chain locus. Like the murine C_δ, the human C_δ is located 85 kb upstream of the C_α gene (31). C_α and C_δ have almost identical intron–exon organizations and a high similarity of sequences within their transmembrane regions, suggesting that they may have arisen by gene duplication. Similar duplications of the V_α gene segments have been observed (32).

Since the D_δ, J_δ, and C_δ genes are located between the V_α and J_α sequences, the possibility of functional rearrangements of the α- and δ-chain genes on the same allele is precluded if the most common rearrangement mechanism, that of looping out and excision, is employed by the T-cell receptor α-chain genes. This mechanism is most likely used in the majority of rearrangements as all of the V_α gene segments located to date are directly upstream of the J_α and C_α genes (32). Furthermore, this organization of the δ- and α-chain genes is consistent with the observation that the δ-chain genes are expressed earlier in thymic ontogeny than the α-chain genes (21) and that the gene products of the δ- and the α-chain genes are found on distinct, and mutually exclusive, subpopulations of T lymphocytes (19, 20). The spacer distribution in the recombinational signals flanking the V_α, V_δ, D_δ, J_α, and J_δ genes allows for sharing of V genes by the two chains, further suggesting a functional relationship between α and δ.

Three J gene segments and two D gene segments can be found within a 40-kb region upstream of the human C_δ locus. Thus, the γ and δ chains have a limited V, D, and J gene repertoire (21, 22, 24, 33), with the majority of the diversity provided by junctional flexibility and N region sequences (34). This concentrates the γδ receptor variability at the VDJ junction of both chains, suggesting that that region may be responsible for binding the polymorphic (V) portion of the target, whereas other γδ residues may bind more constant target residues. At the very least, the concentration of variability points to the VDJ junction as a very important region in the γδ function.

There appears to be only a very limited number of V_δ gene segments and they are exclusively associated with J_δ and C_δ gene sequences, but the reason for this is not known (21, 22, 24, 34). The finding that the spacer distribution in recombinational signals adjacent to V_α segments and the D_δ and J_δ segments allows association among these segments suggests that specific mechanisms are employed to ensure that distinct V_δ gene segments are associated with the δ-chain genes. One possibility for this mutually exclusive usage of V gene segments between the α- and δ-chain genes may be the physical constraints. For example, it is possible that within T cells that utilize the δ-chain genes, V_α, J_α, and C_α genes are inaccessible to the recombinase system (e.g., in a closed chromatin configuration). Another possibility may be the

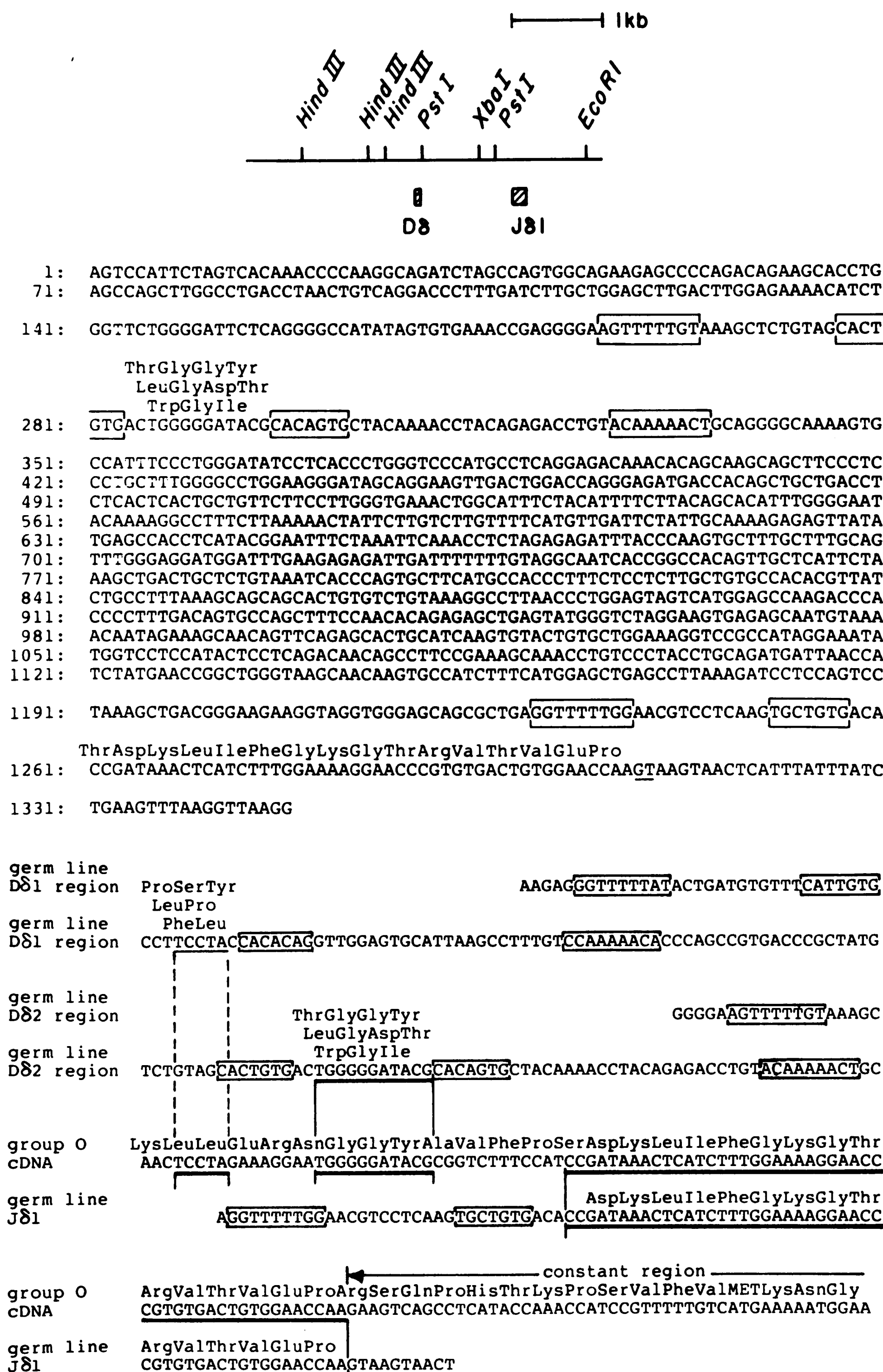

FIG. 4. (*Upper*) Restriction enzyme map and nucleotide sequence of the $D_\delta2$ and $J_\delta1$ region. The genomic map encompassing this region is shown in Fig. 1. The conserved heptamer and nonamer signal sequences are boxed. $D_\delta2$ and $J_\delta1$ regions have the translated amino acid sequence shown above them. (*Lower*) Comparison of germ-line $D_\delta1$, $D_\delta2$, and $J_\delta1$ region sequence with the group O T-cell receptor δ-chain cDNA sequences (22). Sequence homology is denoted by solid lines.

active selection during ontogeny of cells bearing γδ receptors that use only V_δ gene segments.

The data presented here provide the basis for further investigation into δ-chain recombination and into the mechanisms by which T cells "switch" from the δ-chain genes and those of the α chain during thymic ontogeny. Sequences that mediate this switch may be similar to the κ deletion elements found within the murine and human κ-chain genes. Recently certain sequences have been found flanking the δ-chain genes that may be involved in the active transcription of α-chain genes (35) and may also play a part in the transition between δ- and α-chain T-cell receptor use. Furthermore, the elucidation of the organization and sequences surrounding this δ-chain gene will also aid in the study of cells from patients with lymphoid leukemias and lymphomas. We have recently shown that the δ-chain genes, unlike the γ-chain genes, are useful markers for clonality and lineage in a variety of lymphoid malignancies (36). In addition, we have found that this locus is involved in at least three different chromosome translocations (E.C. and M.M., unpublished result).

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