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Immunology

Genomic organization of the human T-cell antigen-receptor α/δ locus

(*TCRA/TCRD* locus/gene rearrangement/nucleotide sequence/cosmid map/field-inversion gel electrophoresis)

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ABSTRACT **Two clusters of overlapping cosmid clones comprising about 100 kilobases (kb) at the human T-cell antigen-receptor α/δ locus were isolated from a genomic library. The structure of the germ-line $V_\delta 1$ variable gene segment was determined. $V_\delta 1$ is located 8.5 kb downstream of the $V_\alpha 13.1$ gene segment, and both V segments are arranged in the same transcriptional orientation. The $V_\alpha 17.1$ segment is located between $V_\delta 1$ and the D_δ, J_δ, C_δ region (containing the diversity, joining, and constant gene segments). Thus, V_δ and V_α segments are interspersed along the chromosome. The germ-line organization of the $D_\delta 2$, $J_\delta 1$, and $J_\delta 2$ segments was determined. Linkage of C_δ to the J_α region was established by identification of J_α segments within 20 kb downstream of C_δ. The organization of the locus was also analyzed by field-inversion gel electrophoresis. The unrearranged $V_\delta 1$ and D_δ, J_δ, C_δ regions are quite distant from each other, apparently separated by a minimum of 175–180 kb.**

In addition to T-cell antigen receptor (TCR) $\alpha\beta$ (a heterodimer of polypeptides termed α and β), a second TCR, $\gamma\delta$, has recently been identified (1). Whereas TCR $\alpha\beta$ is expressed on the majority of peripheral blood T lymphocytes, TCR $\gamma\delta$ is expressed on a small fraction of peripheral blood T lymphocytes, as well as on some thymic T cells and dendritic epidermal cells. The function of the lymphocytes that bear TCR $\gamma\delta$ is unknown.

The genes encoding the TCR α, β, γ, and δ polypeptides are all assembled from multiple gene segments that rearrange to form a functional gene during T-cell differentiation (1–3). The diversity of TCR $\alpha\beta$ is immense due to the use of large numbers of variable (V) and joining (J) segments, and in the case of TCR β, diversity (D) segments as well. TCR $\gamma\delta$ apparently displays a more limited repertoire of germ-line V and J elements but nevertheless displays extraordinary diversity at the V–J junction. This is due in part to the novel use of two D_δ elements that can be incorporated together into the junctional region (4–6).

Whereas the TCR α, β, and γ genes are all unlinked, studies in mice (7), as well as preliminary studies in humans (8–10), indicate that the TCR δ gene lies within the TCR α locus, upstream of the estimated 50–100 J_α segments and between V_α and J_α. However, rearrangement at this locus appears to be highly regulated. Whereas TCR δ genes rearrange early in thymic ontogeny, TCR α genes rearrange much later. Further, the utilization of V segments appears to be selective. For example, to date the human $V_\delta 1$ segment has only been observed to be utilized in TCR $\gamma\delta$ lymphocytes, whereas V_α segments have not been found to be similarly utilized. The details of the organization of the TCR α/δ locus may shed light on the manner in which rearrangements at this locus are controlled. To better understand the structure of the germ-line elements that contribute to the diversity of TCR δ, as well as the organization of these elements with respect to those of TCR α, we have undertaken an analysis of the germ-line organization of the TCR α/δ locus.§

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METHODS

Isolation and Characterization of Genomic Clones. A cosmid library from the human homozygous B-lymphoblastoid cell line MANN has been described (11). Random-primed DNA probes (12) included TCR δ cDNA clones O-240 (specific for the constant segment C_δ) (8) and O-240/38 ($VDJC_\delta$) (8) and 142-base-pair (bp) *Eco*RV–*Nae* I (V_α) and 57-bp *Nae* I–*Eco*RV (J_α) fragments of cDNA L17α (13). $J_\delta 1$, $J_\delta 2$, and $D_\delta 2$ oligomers were labeled by using polynucleotide kinase and [γ-^{32}P]ATP (14). Gel-purified DNA fragments were subcloned into Bluescript plasmid (Stratagene, La Jolla, CA), and sequences were determined on both strands (except where noted) by the dideoxy chain-termination method (15) using double-stranded templates and exonuclease III-generated deletions (refs. 16 and 17; Promega Biotec, Madison, WI).

Field-Inversion Gel Electrophoresis (FIGE). DNA samples were prepared in agarose blocks by modifications of previous procedures (18). After digestion of DNA with restriction endonucleases, FIGE (19) was for 36 hr in a 295 × 135 × 3-mm vertical 1% agarose gel (containing 45 mM Tris, 45 mM boric acid, 1 mM EDTA, and 0.3 μg of ethidium bromide per ml), with a PC 750 pulse controller (Hoefer, San Francisco). The gel was UV-irradiated and the DNA was transferred by electroblotting onto a Hybond-N membrane (Amersham) and UV-crosslinked to the membrane as described by the manufacturer.

RESULTS

Isolation and Characterization of Cosmids Containing V_δ and C_δ Gene Segments. A genomic cosmid library from the homozygous human lymphoblastoid cell line MANN (11) was screened by colony hybridization with a labeled $VDJC_\delta$ cDNA probe (8). Restriction enzyme digests of DNA prepared from those cosmids spanning the largest distances in the V_δ and C_δ regions were electrophoresed in 1% agarose and blots were prepared. Restriction maps were constructed

Abbreviations: TCR, T-cell antigen receptor; FIGE, field-inversion gel electrophoresis; V, D, J, and C are variable, diversity, joining, and constant gene segments, respectively.

§The sequences reported in this paper are being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession nos. J04094 and J04095).

based upon ethidium bromide staining as well as blot hybridization with available cDNA fragments and synthetic oligonucleotides (data not shown) and are presented in Fig. 1. The depicted orientation of the V_δ cosmids relative to the C_δ cosmids is supported by genomic rearrangement data presented in Fig. 3.

$V_\delta 1$ Genomic Segment. The $V_\delta 1$ segment was initially localized to a 10-kb *Xba* I fragment and was fine-mapped within this fragment to a 3-kb *Eco*RI fragment (Fig. 2*A*). The sequence spanning the $V_\delta 1$ segment is presented in Fig. 2*B*. The intron–exon organization was determined by comparison with available cDNA sequences (8). As for other TCR *V* segments, the $V_\delta 1$ segment is composed of two exons, one encoding the majority of the leader sequence and the second encoding the remainder of the leader sequence and the majority of the *V* domain. These exons are separated by a 223-bp intron that displays conserved splice donor and acceptor sequences. Heptamer and nonamer recombination signals separated by 23 bp flank the 3′ end of the coding region.

V_α Segments Lie Upstream and Downstream of $V_\delta 1$. Blots carrying digests of $V_\delta 1$ cosmids were examined at low stringency with V_δ and V_α probes to study the possible association of additional *V* segments within the 45-kb region surrounding $V_\delta 1$. A single weakly hybridizing 3.6-kb *Xba* I–*Asp*718 fragment was identified in cosmid K3A by using a *V* fragment of cDNA L17α (13) ($V_\alpha 17.1$ in the nomenclature of ref. 20) as a probe. This fragment was subcloned and fine-mapped, and the nucleotide sequence was determined (Fig. 2*C*). The sequence extending from the *Asp*718 site toward $V_\delta 1$ revealed an open reading frame representing the 3′ portion of a *V* segment, identified as $V_\alpha 13.1$ based upon 100% nucleotide identity with a published cDNA sequence (20). This *V* segment lies roughly 8.5 kb 5′ of $V_\delta 1$, and both segments are organized in the same transcriptional orientation. Notably, the heptamers flanking $V_\alpha 13.1$ and $V_\delta 1$ are identical, and the nonamers differ at only two positions.

By analyzing Southern blots of *Xba* I-digested genomic DNA samples from TCR $\gamma\delta$ cell lines, we identified one cell line, F7, that displays $V_\delta 1$-to-$(D$–$D)J_\delta 1$ rearrangement on both chromosomes. As shown in Fig. 3, whereas IDP2 cells display two distinct $J_\delta 1$ rearrangements, one of 6.2 kb to $V_\delta 1$ (6) and one of 2.7 kb presumed to be *D*–*J* or *D*–*D*–*J*, the cell line F7 displays only a 6.2-kb $J_\delta 1$ rearrangement. Since F7 retains no germ-line copies of $J_\delta 1$ and has not rearranged $J_\delta 2$, it must carry two rearrangements of $V_\delta 1$ to $J_\delta 1$, a conclusion supported by the intensity of hybridization with the $J_\delta 1$ probe. Further analysis reveals that both IDP2 and F7 retain two copies of $V_\alpha 13.1$ but have deleted one copy and two copies, respectively, of a genomic segment mapping just 3′ to $V_\delta 1$ (Fig. 3). This indicates that $V_\delta 1$ rearrangement occurs by deletion and orients $V_\alpha 13.1$, $V_\delta 1$ and D_δ, J_δ, C_δ as shown in Fig. 1. Strikingly, the $V_\alpha 17.1$ gene segment, like the segment mapping 3′ of $V_\delta 1$, has been deleted on one chromosome in IDP2 and on both chromosomes in F7 (Fig. 3). These results indicate that $V_\alpha 17.1$ lies 3′ of $V_\delta 1$, between $V_\delta 1$ and D_δ, J_δ, C_δ, and provides evidence for the interspersion of V_α and V_δ gene segments.

D_δ, J_δ, and C_δ Segments. Hybridization with a C_δ-specific cDNA probe (8) localized the C_δ gene segment to a 9.4-kb *Xba* I fragment in cosmids K7A and K3B. The $J_\delta 1$ and $J_\delta 2$ segments were mapped by using synthetic oligonucleotides whose design was based upon putative *J* sequences identified in various cDNA clones (6). $J_\delta 2$ was localized to a 1.1-kb *Bam*HI–*Xba* I fragment, present in both K7A and K3B, that mapped 1.5 kb upstream of the fragment carrying C_δ. $J_\delta 1$ was localized to a 1.7-kb *Xba* I fragment, present only in K3B, that mapped about 8 kb further upstream. The sequences spanning the $J_\delta 1$ and $J_\delta 2$ segments are presented in Fig. 4 *A* and *B*. Both J_δ segments are flanked by heptamer and nonamer elements separated by 12 bp at their 5′ ends and by conserved splice donor sites at their 3′ ends. Homology between the two *J* segments is low. Notably, $J_\delta 2$ displays an unusual substitution relative to the core of amino acids that are highly conserved in *J* segments (Phe-Gly-Xaa-Gly-Ile rather than Phe-Gly-Xaa-Gly-Thr). Furthermore, the nonamer element flanking this *J* segment is particularly divergent from the consensus nonamer sequence. These observations may explain why this J_δ segment appears to be utilized less frequently than $J_\delta 1$ (ref. 6 and M.S.K., unpublished observations).

A comparison of the junctional sequences of a panel of human TCR δ cDNA clones revealed two conserved sequences suggestive of the use of two germ-line D_δ elements (6), as has been demonstrated for murine TCR δ (4, 5). By using a synthetic oligonucleotide probe, the putative 3′ *D* element ($D_\delta 2$) was localized to a 3.9-kb *Xba* I fragment that mapped immediately 5′ to the *Xba* I fragment carrying $J_\delta 1$. Nucleotide sequence analysis identified this D_δ element of 13 bp as nucleotides 3525–3537 of the fragment (Fig. 4*C*). This element is flanked by heptamer and nonamer elements separated by 12 bp at its 5′ end and by 23 bp at its 3′ end. Comparison with available cDNA sequences revealed that the $D_\delta 2$ element can be used in multiple translational reading frames (6). The putative human $D_\delta 1$ genomic element has yet to be characterized.

Linkage of the C_δ and J_α Regions. By using a small cDNA fragment carrying J_α sequences as a probe, a 1.6-kb *Xba* I–*Sal* I fragment of K7A that mapped roughly 20 kb downstream of C_δ was identified. The sequence of this fragment revealed two typical J_α segments separated by about 900 bp (data not

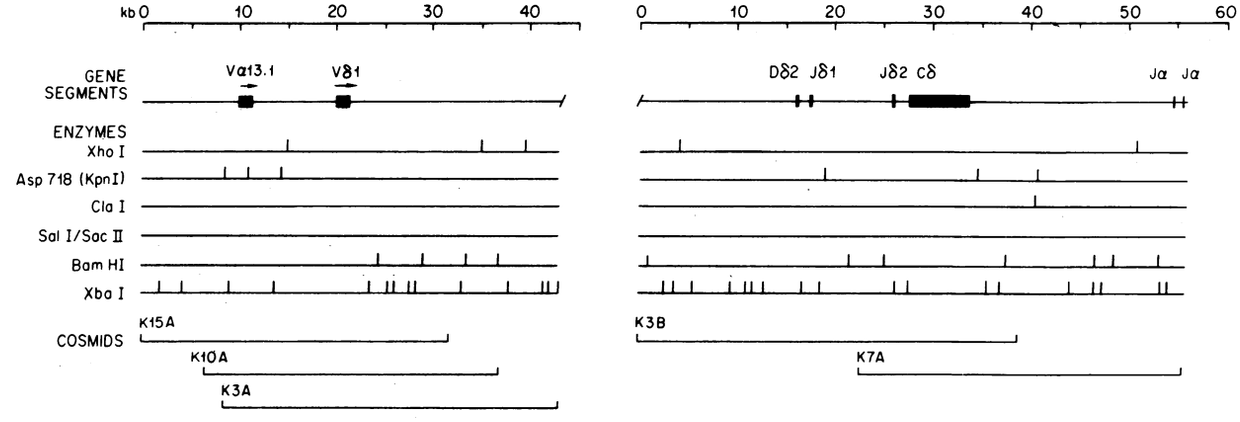
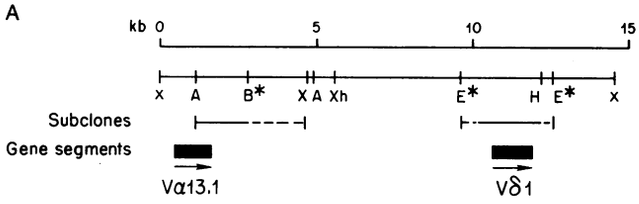


FIG. 1. Cosmid maps of the TCR α/δ locus. (*A*) $V_\delta 1$ region. (*B*) C_δ region. Black boxes represent gene segments. Arrows show the direction of transcription. Note that the placement of some of the small (1-kb) *Xba* I fragments remains tentative. kb, Kilobases.



B

$V_\delta 1$

```
1   CACCGCAGGGCCCTGGTAGGCACTGAACTTTGAGCTTCAGGCAGCACAACTCACATTTGTGCAAAGAGCT
        M  L  F  S  S  L  L  C  V  F  V  A  F  S  Y  S
71  ACATGCCACATGCTGTTCTCCAGCCTGCTGTGTGTATTTGTGGCCTTCAGCTACTCTGGTATGGATGGTT
141 CCAAGTGTGAGTAAAAATGTTAAGGATGGAAGGATTCCAAACAGGCATATGGTATTTCTCATGGACTTGA
211 GGAGGAAAGGAGAAATTGTAAAAAAGCGAGCATATTATGATTTTAGTTTGGTTTGTCTCTGGGTCCTAAA
281 TTAGTCTAGCTCTTACGATATTTTATTCACTATATCAAAGGATCAGCTCTGTTTTCTGATTTTTCCCACA
        G  S  S  V  A  Q  K  V  T  Q  A  Q  S  S  V  S  M  P  V  R  K  A  V
351 GGATCAAGTGTGGCCCAGAAGGTTACTCAAGCCCAGTCATCAGTATCCATGCCAGTGAGGAAAGCAGTCA
        T  L  N  C  L  Y  E  T  S  W  W  S  Y  Y  I  F  W  Y  K  Q  L  P  S  K
421 CCCTGAACTGCCTGTATGAAACAAGTTGGTGGTCATATTATATTTTTTGGTACAAGCAACTTCCCAGCAA
        E  M  I  F  L  I  R  Q  G  S  D  E  Q  N  A  K  S  G  R  Y  S  V  N
491 AGAGATGATTTTCCTTATTCGCCAGGGTTCTGATGAACAGAATGCAAAAAGTGGTCGCTATTCTGTCAAC
        F  K  K  A  A  K  S  V  A  L  T  I  S  A  L  Q  L  E  D  S  A  K  Y
561 TTCAAGAAAGCAGCGAAATCCGTCGCCTTAACCATTTCAGCCTTACAGCTAGAAGATTCAGCAAAGTACT
        F  C  A  L  G  E
631 TTTGTGCTCTTGGGGAACTCACAGTGTTTGAAGTGATAGTAAAAGCAAAACAAAAACCCTAGGGCTCAAT
701 AAGAGAACCCCTCTACTCCCCATCCTTTGCTACAGGAGCCAATCTGAAATGCACACCTGCAGATCTCAGG
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C

$V_\alpha 13.1$

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        Y  Q  Q  F  P  G  K  G  P  A  L  L  I  A  I  R  P  D  V  S  E  K  K
1   GGTACCAACAATTCCCTGGGAAAGGCCCTGCATTATTGATAGCCATACGTCCAGATGTGAGTGAAAAGAA
        E  G  R  F  T  I  S  F  N  K  S  A  K  Q  F  S  L  H  I  M  D  S  Q
71  AGAAGGAAGATTCACAATCTCCTTCAATAAAAGTGCCAAGCAGTTCTCATTGCATATCATGGATTCCCAG
        P  G  D  S  A  T  Y  F  C  A  A  R
141 CCTGGAGACTCAGCCACCTACTTCTGTGCAGCAAGACACAGTGCTCCCCAGGCACCTGAAGCCTGTACCC
211 AAACCTGCAGTTGAGGTTCCAGCCAAACCCCACAGTGGGAGCTTACGTAGGCAGAGATGTAGCCTAGTTT
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FIG. 2. (*A*) Fine map of the $V_\alpha 13.1$–$V_\delta 1$ region. Restriction enzyme sites: A, *Asp*718; B, *Bgl* II; X, *Xba* I; Xh, *Xho* I; E, *Eco*RI; H, *Hin*dIII. Asterisks denote those restriction sites mapped only on the subclones. Solid lines and broken lines denote regions of the subclones whose sequences were determined or not determined, respectively. (*B*) Sequence of the $V_\delta 1$ segment. A portion of the sequence of the 3-kb *Eco*RI fragment is presented. Splice donor and acceptor sequences and heptamer and nonamer recombination signals are underlined. Numbering is from the beginning of the region presented. Predicted amino acid sequences are given in standard one-letter code above the nucleotide sequence. (*C*) Sequence of the V_α segment. The sequence of a portion of the *Asp*718–*Bgl* II fragment extending 3′ from the *Asp*718 site within the coding region is presented. The sequence of only one strand was determined. Numbering is from the 5′ end of the fragment.

shown). It is possible that other J_α segments lie between these segments and C_δ.

FIGE. The relative organization of V_α, V_δ, C_δ, and C_α segments within the TCR α/δ locus was further investigated by FIGE. High molecular weight genomic DNA obtained from cells displaying the germ-line configuration at this locus and cells displaying $VDDJ_\delta$ rearrangements were examined. The C_α and C_δ gene segments were localized to the same 260-kb germ-line *Sal* I fragment in JY and IDF (Fig. 5*A*), whereas the $V_\delta 1$ segment was localized to a distinct *Sal* I fragment of 375 kb in the same cell lines. In addition, a higher molecular weight *Sal* I fragment was reproducibly detected, by using a $V_\delta 1$ probe, in IDF DNA but not in JY DNA (Fig. 5 *A* and *C*); this fragment might be a partial digestion product. By contrast, the C_α, C_δ, and $V_\delta 1$ segments were all localized to distinct *Sfi* I fragments in JY DNA (Fig. 5*B*). The C_α probe detected two fragments, of 60 kb and 20 kb, due to the presence of an *Sfi* I site within one intron of the C_α gene segment (21). The C_δ probe detected an *Sfi* I fragment of

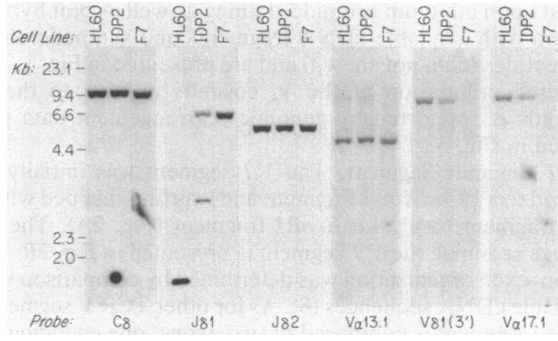

FIG. 3. A V_α segment 3′ of $V_\delta 1$. *Xba* I-digested genomic DNA samples (5 μg) from the cell lines HL60 (myeloid), IDP2 (TCR γδ) and F7 (TCR γδ) were electrophoresed in 0.7% agarose, blotted onto a Hybond-N membrane, and analyzed by hybridization with the indicated probes. Probes were C_δ (O-240; ref. 8), $J_\delta 1$ (1.7-kb *Xba* I fragment of K3B), $J_\delta 2$ (1.1-kb *Bam*HI–*Xba* I fragment of K3B), $V_\alpha 13.1$ (220-bp *Asp*718–*Pst* I fragment carrying the sequenced portion of the coding region), $V_\delta 1$(3′) (300-bp *Hin*dIII–*Eco*RI fragment mapping just 3′ to the coding region; see Fig. 2*A*), and $V_\alpha 17.1$ (340-bp *Pst* I–*Bam*HI fragment of Lα17; ref. 13). A single blot was serially analyzed with all probes. A phage λ *Hin*dIII digest served as size markers.

roughly 180 kb, whereas the $V_\delta 1$ probe detected a fragment of 190 kb. These fragments were clearly distinguishable based on double digestion with *Sal* I plus *Sfi* I (Fig. 5*B*). These data are summarized in the map of the unrearranged chromosome presented in Fig. 5*D*.

Two V_α segments, $V_\alpha 17.1$ and $V_\alpha 12.1$ (22) were localized to the same *Sal* I and *Sfi* I fragments as $V_\delta 1$ (Fig. 5*C* and data not

A

$J_\delta 1$

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421 AAGCAAACCTGTCCCTACCTGCAGATGATTAACCATCTATGAACCGGCTGGGTAAGCAACAAGTGCCATC
491 TTTCATGGAGCTGAGCCTTAAAGATCCTCCAGTCCTAAAGCTGACGGGAAGAAGGTAGGTGGGAGCAGCG
                                                T  D  K  L  I  F  G  K  G  T  R  V
561 CTGAGGTTTTTGGAACGTCCTCAAGTGCTGTGACACCGATAAACTCATCTTTGGAAAAGGAACCCGTGTG
        T  V  E  P
631 ACTGTGGAACCAAGTAAGTAACTCATTTATTTATCTGAAGTTTAAGGTTAAGGCATCCTCCATCTAAGGA
701 GGCAGAAATAATCCTGAAATGGGAAATGGGTGAAATAGCTAGCATTTAGGAGGACTCCTGGGAAGAGGTG
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B

$J_\delta 2$

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581 GCCCCTTGGTCTCATCAAGAGCAGCTTTGTAGTTCTCTGAGCTGTGGGGTCTCTAGGCTGAGAACTGAGG
651 CTGGGGAGGCAGGGCACAGATGTTACAGCTCAGGCCCCAGGGCCAGCTCCAGGCTAGTTACCTGTGAGGC
                        S  W  D  T  R  Q  M  F  F  G  T  G  I  K  L  F  V  E
721 ACTGTCATAATGTGCTCCTGGGACACCCGACAGATGTTTTTCGGAACTGGCATCAAACTCTTCGTGGAGC
        P
791 CCCGTGAGTTGATCTTTTTCCTATATTTCTGGGATAATTTGAGTCCTGGCACTGGGGCTGCAATCCAGTT
861 TGCATTATAAATTATAATAGTAAATGAAATTATAACAAGGAGACAGAGTATTACAGATGTGAAATAGGCC
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C

$D_\delta 2$

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3361 AGCCCCAGACAGAAGCACCTGAGCCAGCTTGGCCTGACCTAACTGTCAGGACCCTTTGATCTTGCTGGAG
3431 CTTGACTTGGAGAAAACATCTGGTTCTGGGGATTCTCAGGGGCCATATAGTGTGAAACCGAGGGGAAGTT
3501 TTTGTAAAGCTCTGTAGCACTGTGACTGGGGGATACGCACAGTGCTACAAAACCTACAGAGACCTGTACA
3571 AAAACTGCAGGGGCAAAAGTGCCATTTCCCTGGGATATCCTCACCCTGGGTCCCATGCCTCAGGAGACAA
3641 ACACAGCAAGCAGCTTCCCTCCCTGCTTTGGGGCCTGGAAGGGATAGCAGGAAGTTGACTGGACCAGGGA
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FIG. 4. Structure of the $J_\delta 1$, $J_\delta 2$, and $D_\delta 2$ elements. (*A*) Sequence of the $J_\delta 1$ segment. The sequence of the 1.7-kb *Xba* I fragment of cosmid K3B was determined, and the region surrounding $J_\delta 1$ is presented. Heptamer and nonamer recombination signals and a splice donor signal are underlined. Numbering is from the 5′ end of the *Xba* I fragment. (*B*) Sequence of the $J_\delta 2$ segment. The sequence of the 1.1-kb *Bam*HI–*Xba* I fragment of cosmid K3B was determined, and the region surrounding $J_\delta 2$ is presented. Numbering is from the 5′ end of the fragment. (*C*) Sequence of the $D_\delta 2$ element. The sequence of the 3.9-kb *Xba* I fragment of cosmid K3B was determined, and the region surrounding $D_\delta 2$ is presented. Heptamer and nonamer recombination signals are underlined, and the $D_\delta 2$ segment is overlined. Numbering is from the 5′ end of the fragment.

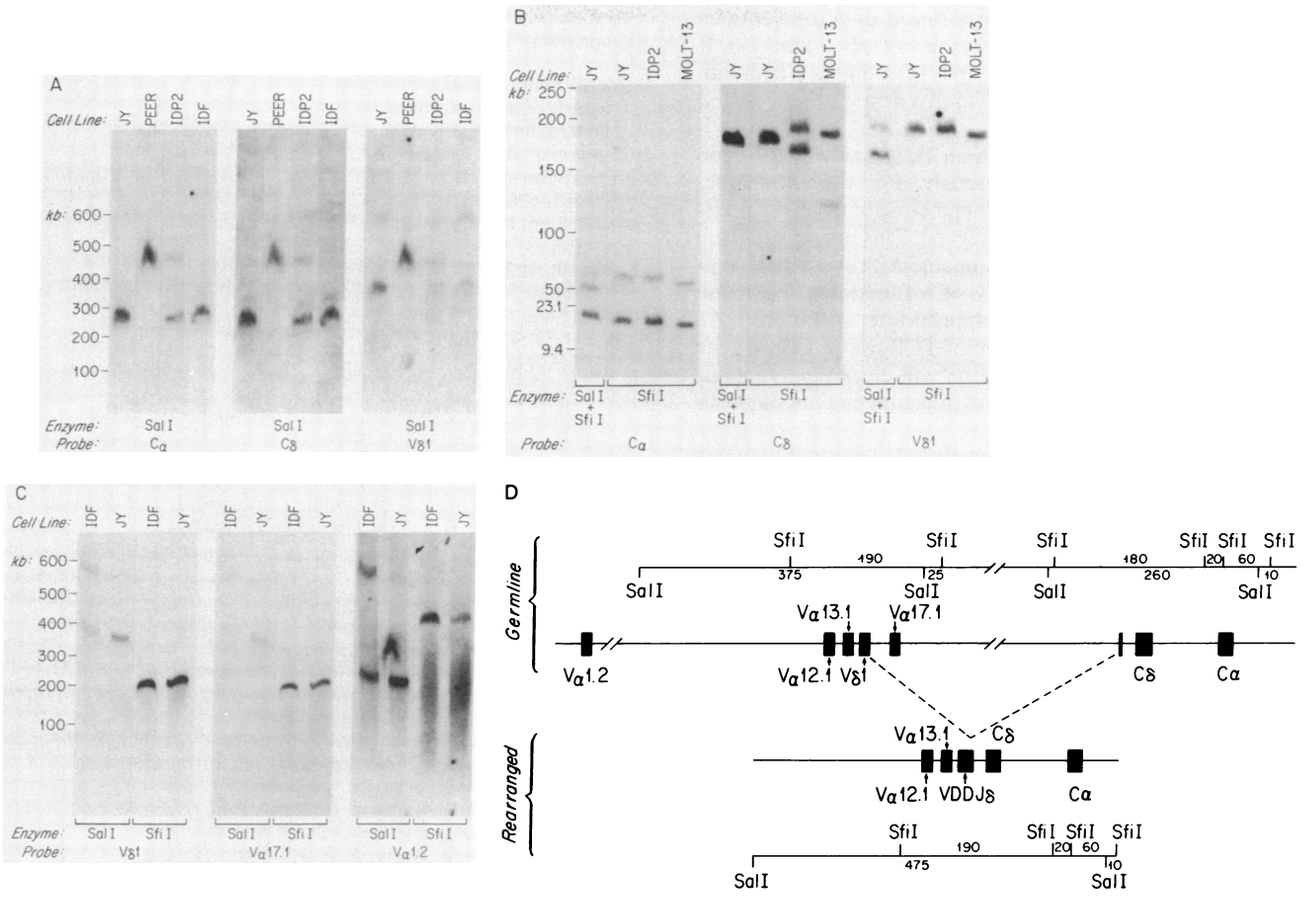

FIG. 5. FIGE analysis. (*A*) *Sal* I digests of JY and IDF DNA (B cells) and PEER and IDP2 DNA (TCR-γδ T cells) were analyzed by FIGE at 200 V, with a ratio of forward to reverse pulse times of 3 and a ramp of forward pulse times from 2.4 sec to 84 sec. A single blot was serially probed with C_α (400-bp *Pvu* II fragment of cDNA L17α), C_δ (O-240), and $V_\delta 1$ (300-bp *Eco*RI–*Sca* I fragment of O-240/47) probes labeled by random priming. Ligated phage λ concatemers served as size markers. (*B*) *Sfi* I- and *Sal* I/*Sfi* I-digested DNA samples were analyzed by FIGE at 167 V, with a ratio of forward to reverse pulse times of 3 and a ramp of forward pulse times from 3 sec to 20 sec. A single blot was analyzed with probes identical to those in *A*. Molt-13 is a TCR-γδ T-cell line. (*C*) *Sal* I- and *Sfi* I-digested DNA samples were analyzed by FIGE as in *A*. A single blot was analyzed with $V_\delta 1$, $V_\alpha 17.1$, and $V_\alpha 1.2$ (500-bp *Bam*HI–*Sca* I fragment of PY14; ref. 23) probes labeled as above. (*D*) Germ-line and rearranged maps of the TCR α/δ locus determined by FIGE. Numbers denote distances in kb.

shown). As described above, deletional analysis mapped $V_\alpha 17.1$ 3′ to $V_\delta 1$ (Fig. 3). Since $V_\delta 1$ is deleted on a chromosome that has rearranged $V_\alpha 12.1$ (data not shown), the latter must map 5′ of the former. Cosmid analysis mapped $V_\alpha 13.1$ immediately 5′ to $V_\delta 1$ (Figs. 1 and 2). One other V_α segment tested, $V_\alpha 1.2$ (23), mapped to distinct *Sal* I and *Sfi* I fragments (Fig. 5*C*). These fragments are presumed to map distal to those carrying $V_\delta 1$, $V_\alpha 17.1$, and $V_\alpha 12.1$, since a chromosome that has rearranged $V_\alpha 1.2$ has deleted $V_\delta 1$ (data not shown). Whether these sets of fragments are linked is not known.

DNA from the TCR γδ cell lines PEER, IDP2, and Molt-13 was analyzed in order to determine the structure of chromosomes carrying a functionally rearranged TCR δ gene. PEER displayed a single *Sal* I fragment of 475 kb that hybridized with C_α, C_δ, and V_δ probes (Fig. 5*A*). IDP2 displayed a rearranged fragment of the same size that hybridized with all three probes. As expected, the rearranged fragment did not hybridize with a $V_\alpha 17.1$ probe (data not shown). In addition, IDP2 displayed apparent germ-line fragments originating from the nonproductively rearranged chromosome (note that the germ-line IDP2 pattern detected by the $V_\delta 1$ probe resembles that of IDF rather than that of JY; Fig. 5*A*). IDP2 and Molt-13 displayed 190-kb *Sfi* I fragments hybridizing with both $V_\delta 1$ and C_δ probes, as well as an additional (nonproductively) rearranged fragment in each cell line detected by the C_δ probe (Fig. 5*B*). These data are summarized in the map of the rearranged chromosome presented in Fig. 5*D*.

These data are insufficient to link the V_δ and C_δ segments in the germ-line configuration. However, given that rearrangement of $V_\delta 1$ to D_δ–J_δ occurs by deletion (Fig. 3), it is possible to estimate the minimal distance between these segments. If the *Sfi* I fragments carrying $V_\delta 1$ (190 kb) and C_δ (180 kb) were adjacent to each other, the detection of a rearranged *Sfi* I fragment of 190 kb would imply the deletion of 180 kb in the process of rearrangement (190 + 180 − 190 = 180). Similarly, the detection of a rearranged *Sal* I fragment of 475 kb would predict the deletion of 175 kb in the process of rearrangement (375 + 25 + 260 − 10 − 475 = 175). Thus, $V_\delta 1$ lies a minimum of 175–180 kb away from $J_\delta 1$. However, since the *Sfi* I fragments in question may not be adjacent in the germ-line configuration, this distance could be greater.

DISCUSSION

The results of these and other studies indicate that the human TCR δ gene segments are nested within the TCR α locus on human chromosome 14. Within this locus are 50–100 V_α segments, 50–100 J_α segments, one or more V_δ segments, two D_δ segments, and two J_δ segments, all of which take part in recombinational events leading to the assembly of functional TCR α and TCR δ genes. In contrast to TCR α, there are only a limited number of TCR δ germ-line segments. The diversity of TCR δ is nevertheless high, because the use of multiple *D*

elements and the extensive incorporation of *N* nucleotides generates tremendous variability at the *V–D–D–J* junction. It is rather striking that the TCR α and TCR δ genes maintain distinct strategies to generate diversity despite their nested chromosomal arrangement.

Results to date suggest that human TCR α and TCR δ gene segments are not used promiscuously. For example, functional rearrangements of $V_\delta 1$ to J_α segments, or of V_α segments to D_δ segments, have not been observed. This may result from control at the level of the rearrangement process *per se* or perhaps at the level of assembly of a functional, heterodimeric receptor ($\alpha\beta$ or $\gamma\delta$) of appropriate specificity. An understanding of the organization of TCR α and TCR δ gene segments within the TCR α/δ locus will clearly be valuable in understanding whether and how rearrangements might be controlled.

It is a striking finding of this study that $V_\delta 1$ is situated within 8.5 kb of a known V_α segment, $V_\alpha 13.1$, yet at quite some distance from D_δ, J_δ, C_δ. Although we have not determined this distance directly, our data imply that $V_\delta 1$ and D_δ, J_δ, C_δ are separated by a minimum of 175–180 kb. Further, at least one V_α segment, $V_\alpha 17.1$, lies between $V_\delta 1$ and D_δ, J_δ, C_δ. Thus V_α and V_δ segments are not segregated from each other and in fact are interspersed within the locus. Murine V_α and V_δ segments may be similarly interspersed (5).

Since $V_\delta 1$ rearrangements, but not $V_\alpha 13.1$ and $V_\alpha 17.1$ rearrangements, to D_δ, J_δ, C_δ have been detected in TCR $\gamma\delta$ lymphocytes, *V*-segment utilization cannot be controlled by proximity to D_δ, J_δ, C_δ and/or the gross organization of the *V* segments on the chromosome. Proximity has been invoked to explain the hierarchy of rearrangements at the immunoglobulin heavy-chain locus (24). In addition, specificity is unlikely to be provided by the recombination signals flanking the *V* segments, since those flanking $V_\delta 1$ and $V_\alpha 13.1$ are virtually identical to each other, suggesting the use of a common recombination machinery (25). If control of rearrangement is mediated through modulation of the accessibility of particular segments of the chromosome, as has been suggested (26, 27), the data presented in this paper would imply that such accessibility is limited to within 8 kb 5′ of $V_\delta 1$. Alternatively, there may be other elements involved in regulating the recombinational events within the locus. Further analysis will be required to assess the extent of the germ-line human TCR V_δ repertoire, as well as the details of the organization and control of rearrangement of V_α and V_δ segments within the TCR α/δ locus. Functional studies of TCR $\gamma\delta$ lymphocytes may, on the other hand, provide the rationale for this complex organization and control.

Note Added in Proof. Since the submission of this manuscript another J_δ segment has been described that maps to the genomic region between the two J_δ segments characterized here (28, 29). As such, the 3′ J_δ segment identified here as $J_\delta 2$ should be renamed $J_\delta 3$. Our probe should detect rearrangements to both $J_\delta 2$ and $J_\delta 3$.

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