DIVERSITY AND ORGANIZATION OF HUMAN T CELL RECEPTOR δ VARIABLE GENE SEGMENTS

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Two CD3-associated TCR complexes have now been identified. The first, TCR-α/β, is expressed on the majority of T lymphocytes. TCR-α and -β polypeptides mediate the specific recognition of antigen in association with MHC-encoded class I and class II molecules (1). TCR-α/β diversity is high, since in man there are 50–100 V_α and 50–100 J_α gene segments, as well as 70 V_β, 13 J_β, and two D_β gene segments (2, 3). Combinatorial and imprecise joining, polymerization of template independent N region nucleotides, and combinatorial association of TCR-α and -β polypeptides help to generate an extensive TCR-α/β repertoire.

The second, TCR-γ/δ, is expressed on a small fraction of peripheral blood and thymic T lymphocytes (4–7), although it may be expressed on the majority of T lymphocytes in certain epithelial locations (8, 9). The function of TCR-γ/δ lymphocytes is unclear at present, although one example of murine TCR-γ/δ lymphocytes that recognize an MHC-linked gene product has been reported (10). The repertoire of germline TCR-γ and -δ gene segments is apparently more limited than that of TCR-α and -β. In man, for example, there are only seven functional V_γ segments and five J_γ segments, with additional diversity introduced at the V-J junction due to N region nucleotide incorporation (7, 11–15). Similarly, initial studies have identified only a single human V_δ segment ($V_\delta1$) and three J_δ segments ($J_\delta1$, $J_\delta2$, and $J_\delta3$), with all productive rearrangements involving the $J_\delta1$ segment (16–18). The TCR-γ/δ repertoire is nevertheless likely to be large, since the use of two D_δ elements ($D_\delta1$ and $D_\delta2$) in tandem, coupled with imprecise joining and extensive N region nucleotide incorporation, generates marked variability in the TCR-δ V-J junctional region (18–23).

The location of the human TCR-δ gene is unusual in that it is nested within the TCR-α locus at chromosome 14q11 (19, 20, 23–26). The D_δ, J_δ, and C_δ gene segments are located just upstream of the J_α region, between V_α and J_α segments. It is striking that although the $V_\delta1$ segment is located among V_α segments and at a large distance from C_δ (20), the $V_\delta1$ segment appears to rearrange selectively to D_δ-J_δ. This suggests that the V_α and V_δ repertoires may be distinct, and that rearrangement at the TCR-α/δ locus may be highly controlled. By contrast, as many

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as nine murine V_δ gene segments have now been identified (22). Two of these are nearly identical to previously characterized V_α gene segments, indicating the possibility that some V_δ segments may function as V_α segments as well.

To better understand the diversity of human TCR-δ gene segments and the relationship between the TCR-γ/δ and TCR-α/β repertoires, we have examined TCR-δ rearrangements in a new panel of cloned TCR-γ/δ lymphocytes. Through this analysis we have identified and determined the structures of two new V_δ segments, designated $V_\delta2$ and $V_\delta3$. These segments are distinct from known V_α segments, and are widely separated within the TCR-α/δ locus both from $V_\delta1$ and from each other.

Materials and Methods

TCR-γ/δ Cell Lines. The derivation and characteristics of peripheral blood-derived cell line IDP2 (4) and leukemic cell line Molt-13 (17, 27) have been described previously. The cloned T cell line WM-14 was derived from umbilical cord blood cells (28). The T cell clones LB117, LB207, LB210, LB213, and LB220 were obtained by stimulating the PBL from a healthy donor with Burkitt's lymphoma cells, followed by cloning using limiting dilution and feeder cell mixtures as described previously (29). The derivation and properties of T cell clone F7 will be described in a subsequent publication. The T cell clones were maintained in Yssel's medium and were expanded in IL-2 by weekly stimulation with feeder cells (29).

DNA Probes. The TCR-δ DNA probes used in this study were as follows: C_δ, cDNA O-240 (16); $J_\delta1$, a 1.7-kb genomic Xba I fragment (18, 20); $J_\delta3$, a 1.1-kb Bam HI-Xba I genomic fragment (previously called $J_\delta2$) (18, 20); $V_\delta1$, a 300-bp Eco RI-Sca I fragment of cDNA O-240/47 (16); $V_\delta2$, a 430-bp Eco RI-Nco I fragment of cDNA LB117δ1.7; and $V_\delta3$, a 435-bp Eco RI-Taq I fragment of cDNA WM14δ2.1. The TCR-γ DNA probes used in this study were as follows: $J_\gamma1.3/2.3$, an 800-bp $J_\gamma1.3$ genomic Hind III-Eco RI fragment (14); $V_\gamma1$, a 350-bp Eco RI-Kpn I fragment of $V_\gamma1.3$ cDNA M13k (27); $V_\gamma2$, a 445-bp Eco RI-Acc I fragment of cDNA PBLC1.15 (30); $V_\gamma3$, a 300-bp Eco RI fragment of cDNA $pT_\gamma15$ (W. Strauss, personal communication); $V_\gamma4$, a 400-bp Ssp I-Eco RI fragment of rearranged genomic clone $pT_\gamma R4$ containing both V and J sequences (W. Strauss, personal communication). Fragments were purified through low gelling temperature agarose and labeled to high specific activity by the random priming method (31).

Preparation and Blot Hybridization Analysis of RNA and DNA Samples. Total cellular RNA and high molecular weight genomic DNA were prepared from a single frozen pellet of ~5 × 10^7 cells in each instance. Cells were lysed for RNA preparation by the urea-lithium chloride method (32). 90% of the lysate was sheared and used for RNA preparation, whereas 10% of the lysate was diluted into 15 vol of 150 mM NaCl; 10 mM NaEDTA; 10 mM Tris HCl (pH 8.0); 0.4% SDS; 100 μg/ml proteinase K, and was used for the preparation of genomic DNA (33). Yields averaged 85 μg RNA and 50 μg DNA.

RNA was subjected to electrophoresis through 1.5% agarose gels containing 2.2 M formaldehyde (34), and was then blotted onto and UV crosslinked to Hybond-N membranes (Amersham Corp., Arlington Heights, IL), according to the manufacturer's instructions. Genomic DNA was digested with the appropriate restriction enzyme, subjected to electrophoresis through 0.7% agarose gels, and was similarly blotted onto and UV crosslinked to Hybond-N membranes. Filters were prehybridized at 50°C and hybridized at 42°C in 50% formamide; 4.8× SSC; 5× Denhardt's; 0.5% SDS; 200 μg/ml boiled salmon sperm DNA; 50 mM Hepes (pH 7.0). High stringency washes were in 0.1× SSC; 0.1% SDS at 50°C for Northern blots and at 60°C for Southern blots. Blots were stripped before reprobing by incubation for 1-2 h at 68°C in the prehybridization solution.

cDNA Library Construction and Analysis. cDNA libraries containing 2-3 × 10^5 recombinants were constructed from LB117 RNA, WM14 RNA, and a mixture of LB207, LB210, and F7 RNA. Poly(A)$^+$ RNA prepared from ~80 μg total RNA was converted to dscDNA using AMV reverse transcriptase, klenow, mung bean nuclease, and Eco RI methylase. After ligation of Eco RI linkers, size-fractionated cDNA was cloned into the Eco RI site of the vector

λgt10 (35). After screening with the appropriate probes, the 5′ Eco RI fragments (VJCδ) of positive clones and subfragments thereof were subcloned into either Bluescript (Stratagene) or M13mp18, and nucleotide sequences were determined on both strands by the dideoxy chain termination method (36, 37) using modified T7 polymerase (38) (Sequenase; United States Biochemical Corp., Cleveland, OH).

Analysis of Genomic Clones. An 11-kb Bam HI–Kpn I fragment carrying the 3′ portion of Vδ3, and a 3-kb Kpn I–Bam HI fragment carrying the 5′ portion of Vδ3 were isolated from cosmid K7A (20) and subcloned into Bluescript. Sequences were determined by the dideoxy chain termination method using double-stranded templates and exonuclease III–generated deletions (39, 40) (Erase-a-base System; Promega Biotec, Madison, WI) according to the manufacturer.

Results

Two Novel Human Vδ Segments. In previous studies it was shown that the TCR-γ/δ peripheral blood T cell lines IDP2, PBL C1, and PBL L1, and the leukemic T cell lines PEER and Molt-13, all displayed functional rearrangements using the Vδ1 and Jδ1 gene segments (16–18). To identify TCR-γ/δ cell lines using distinct Vδ segments, RNA samples from a panel of seven TCR-γ/δ T lymphocyte peripheral blood clones were examined by blot hybridization using Vδ1 and Cδ probes. As in RNA from the previously studied IDP2 cell line (16), a Cδ probe revealed TCR-δ mRNA species indicative of differentially polyadenylated transcripts arising from fully rearranged genes (2.2 and 1.3 kb) in all cell lines (Fig. 1 *A*). In addition, this probe detected differentially polyadenylated transcripts arising from partially or unrearranged genes (1.7 and 0.8 kb) in some cell lines. However, aside from IDP2 RNA, only a single cell line (F7) revealed transcripts (2.2 and 1.3 kb) detected by a Vδ1 probe (Fig. 1 *B*). The remaining cell lines (LB117, LB207, LB210, LB213, LB220, and WM14), which expressed full-length Vδ1$^-$ transcripts of 2.2 and 1.3 kb, were presumed to be using distinct Vδ gene segments.

To determine whether the Vδ1$^-$ cell lines all used the same novel Vδ segment, Xba I digests of genomic DNA samples were analyzed by blot hybridization using Jδ1 and Jδ3 probes (Fig. 2, *A* and *B*). The Jδ1 probe detects a single germline fragment of 1.7 kb in SB (B cell) and HL60 (myeloid cell), and two rearrangements in the TCR-γ/δ cell lines IDP2 and Molt-13. Of these rearrangements, the 6.4-kb fragment shared by IDP2 and Molt-13 represents rearrangement to Vδ1 (18). The 2.9-kb rearrangement in IDP2 is quite common, and is thought to represent either D-J or D-D-J, whereas the 7.2-kb rearrangement in Molt-13 has not been detected elsewhere, and is unidentified. As expected, the Vδ1$^+$ cell line F7 shares the 6.4-kb Vδ1-Jδ1 rearrangement with IDP2 and Molt-13. On the other hand, the Vδ1$^-$ cell lines display three distinct Jδ1 rearrangements, two of which (4.2 and 1.9 kb) had not been observed previously. These were presumed to reflect rearrangement to two novel Vδ segments, designated Vδ2 (4.2 kb) and Vδ3 (1.9 kb).

Based upon the above assignments, neither LB207 nor LB213 carries a functional Jδ1 rearrangement. We have previously described a distinct Jδ segment (Jδ2) that is homologous to the murine Jδ2 element and is located ~10 kb 3′ of Jδ1. Since it is now clear that an additional Jδ segment lies between these two J segments (19, 23), it seems appropriate to rename the most 3′ Jδ segment as Jδ3. Our Jδ3 probe detects a 5.0-kb germline Xba I fragment that carries both Jδ2 and Jδ3, and therefore, should detect rearrangements to either of these Jδ segments. Notably, the Jδ3 probe detects a 3.4-kb rearrangement specific to LB207 and LB213, indicating the

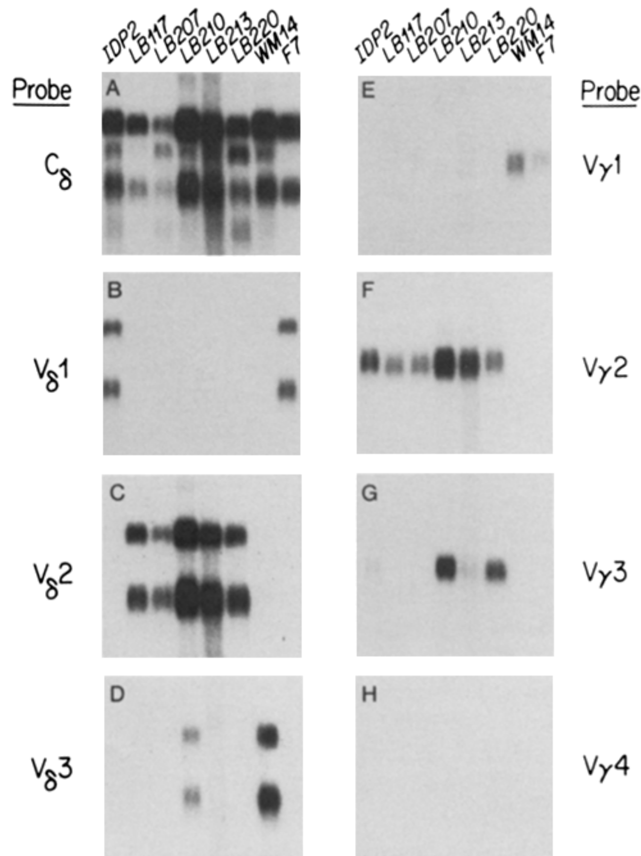

FIGURE 1. Blot hybridization analysis of TCR-δ and TCR-γ transcripts. 5-μg samples of total RNA was subjected to electrophoresis through 1.5% agarose containing 2.2 M formaldehyde, transferred to Hybond-N membranes, and serially probed with the indicated ^{32}P-labeled DNA fragments.

likelihood of functional rearrangement to either Jδ2 or Jδ3 in these cell lines. Based upon the disposition of Xba I sites flanking Jδ1, Jδ2, and Jδ3, we predicted that in these instances Jδ3 had rearranged to the same V segment (Vδ2) identified by the 4.2-kb Jδ1 rearrangement.

To confirm these interpretations and characterize the novel V segments, we generated cDNA libraries from LB117 and from WM14 poly(A)$^+$ RNA. Using a C_δ probe we identified cDNA clones representing the transcripts of the functionally rearranged TCR-δ gene in each cell line. The nucleotide and deduced amino acid sequences of these cDNA clones are presented in Fig. 3, *A* and *B*, and the deduced amino acid sequences for the V segments are compared both with each other and with that of Vδ1 in Fig. 3 *C*. LB117 (Vδ2) and WM14 (Vδ3) clearly use V_δ segments distinct from the Vδ1 segment and from each other. Both cDNAs display potential contributions from the Dδ1 and Dδ2 elements as well as from N region nucleotides within the junctional region, both utilize the Jδ1 segment, and both maintain an open reading frame across the V-D-D-J junction.

The Vδ1, Vδ2, and Vδ3 gene segments are all distinct from previously reported V_α segments, further supporting the notion that the human V_α and V_δ repertoires

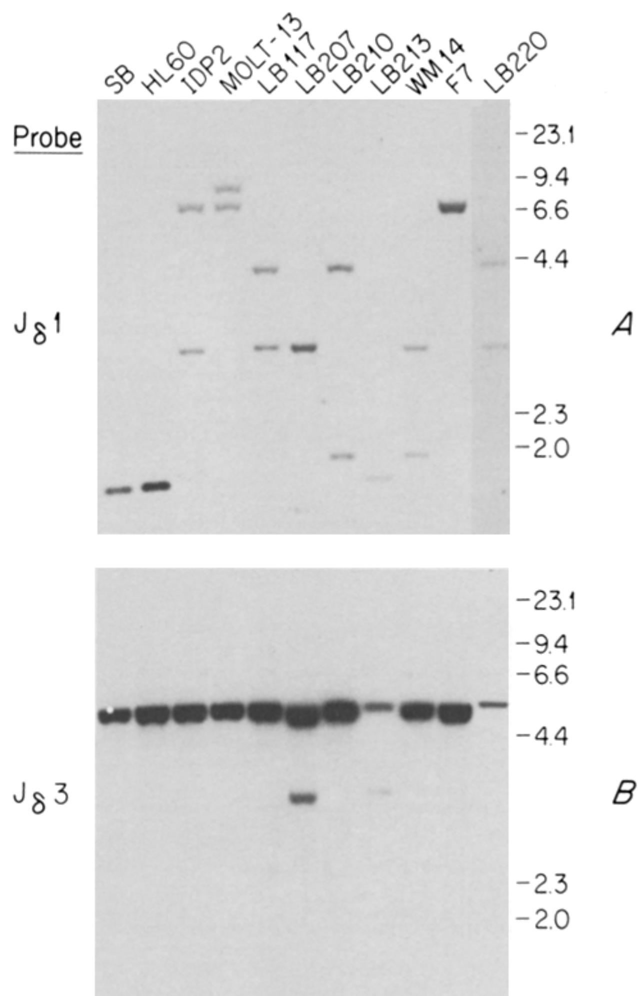

FIGURE 2. Blot hybridization analysis of Jδ rearrangements. Samples of genomic DNA were digested with Xba I, subjected to electrophoresis through 0.7% agarose, transferred to Hybond-N membranes, and serially probed with the indicated ^{32}P-labeled DNA fragments. The amount of DNA loaded was 5 μg in most instances, but was ~2.5 μg for LB213 and ~1 μg for LB220. The data for LB220 in *A* derives from a longer exposure of the autoradiograph than the data for the remaining samples. SB and HL60 served as germline controls. A phage λ Hind III digest served as molecular weight markers.

are distinct. Furthermore, they display only low levels of amino acid sequence identity with each other, matching at 23–33% of the residues in various pairwise comparisons (Fig. 3 *C*). Nevertheless there are striking homologies with particular murine V_δ segments (Fig. 3 *C*). For example, human $V_\delta1$ matches a member of the murine $V_\delta6$ family at 58% of the residues, and human $V_\delta3$ matches murine $V_\delta5$ at 66% of the residues (22). Such high levels of interspecies sequence conservation despite low levels of intraspecies sequence conservation most probably indicate that these pairs of V segments have evolved from distinct ancestral V segments whose existence predates the divergence of mouse and man. Similar observations have been made for particular human and murine V_β (41) and V_γ (42) gene segments.

Rearrangement and Expression of TCR-δ and TCR-γ Gene Segments. To further characterize the TCR-δ rearrangements in the remaining TCR-γ/δ cell lines in the panel, $V_\delta2$- and $V_\delta3$-specific probes were generated and were used to probe both Northern

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A                                            Vδ2
                                                                                                        M  Q  R  I  S  S  L  I  H
LB117    AACACTTGTGTGTTGGTTCAGAGGAGGGACCAGGCAGAAGGTGGTTGAGAGGCAGAGCTGCCCCTGAGTGAGCCATGCAGAGGATCTCCTCCCTCATCCA
LB210                                                                                                                    CTCATCCA
LB207                                                                                                                   CCTCATCCA

          L  S  L  F  W  A  G  V  M  S  A  I  E  L  V  P  E  H  Q  T  V  P  V  S  I  G  V  P  A  T  L  R  C
LB117    TCTCTCTCTCTTCTGGGCAGGAGTCATGTCAGCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGC
LB210    TCTCTCTCTCTTCTGGGCAGGAGTCATGTCAGCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGC
LB207    TCTCTCTCTCTTCTGGGCAGGAGTCATGTCAGCCATTGAGTTGGTGCCTGAACACCAAACAGTGCCTGTGTCAATAGGGGTCCCTGCCACCCTCAGGTGC

          S  M  K  G  E  A  I  G  N  Y  Y  I  N  W  Y  R  K  T  Q  G  N  T  I  T  F  I  Y  R  E  K  D  I  Y
LB117    TCCATGAAAGGAGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATCACTTTCATATACCGAGAAAAGGACATCTATG
LB210    TCCATGAAAGGAGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATCACTTTCATATACCGAGAAAAGGACATCTATG
LB207    TCCATGAAAGGAGAAGCGATCGGTAACTACTATATCAACTGGTACAGGAAGACCCAAGGTAACACAATCACTTTCATATACCGAGAAAAGGACATCTATG

         G  P  G  F  K  D  N  F  Q  G  D  I  D  I  A  K  N  L  A  V  L  K  I  L  A  P  S  E  R  D  E  G  S  Y
LB117    GCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTTGCACCATCAGAGAGAGATGAAGGGTCTTA
LB210    GCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTTGCACCATCAGAGAGAGATGAAGGGTCTTA-
LB207    GCCCTGGTTTCAAAGACAATTTCCAAGGTGACATTGATATTGCAAAGAACCTGGCTGTACTTAAGATACTTGCACCATCAGAGAGAGATGAAGGGTCTTA

                              N    Dδ1   N   Dδ2        N                                  Jδ1
          Y  C  A  C  D              T         M  G    H  P  R  G                    K  L  I  F  G  K  G  T
LB117    CTACTGTGCCTGTGA    CA     CC  A    TGGGG   CACCCACGGGG                  TAAACTCATCTTTGGAAAAGGAACCC

          Y  C  A  C  D    H     T  S      D  W      T  G  N  Q            D  T  D  K  L  I  F  G  K  G  T
LB210    CTACTGTGCCTGTGA  TCATA  CTTCC  G  ACTGG    ACTGGTAATCAGG           ACACCGATAAACTCATCTTTGGAAAAGGAACCC
                                                                                                   Jδ3
          Y  C  A  C  D                R     L  G       V  S          S  W  D  T  R  Q  M  F  F  G  T  G  I
LB207    CTACTGTGCCTGTGA              CC  G  ACTGGGGG      TTAG       CTCCTGGGACACCCGACAGATGTTTTTCGGAACTGGCATCA

                                                           Cδ
         R  V  T  V  E  P    R  S  Q  P  H  T  K  P  S  V  F  V  M  K  N  G  T  N  V  A  C  L  V  K  E  F
LB117    GTGTGACTGTGGAACCAA  GAAGTCAGCCTCATACCAAACCATCCGTTTTTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAATTC
LB210    GTGTGACTGTGGAACCAA  GAAGTCAGCCTCATACCAAACCATCCGTTTTTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAATTC

         K  L  F  V  E  P    R  S  Q  P  H  T  K  P  S  V  F  V  M  K  N  G  T  N  V  A  C  L  V  K  E  F
LB207    AACTCTTCGTGGAGCCCC  GAAGTCAGCCTCATACCAAACCATCCGTTTTTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAATTC


B                                            Vδ3
                                                                                                        M  I  L  T  V  G  F  S  F
WM14                      GAGACTATCTTAGGAACCCTGTTTTGTTGTATATCCTCTGCCCTGATATGAAGAAAAGATGATTCTTACTGTGGGCTTTAGCTT

          L  F  F  Y  R  G  T  L  C  D  K  V  T  Q  S  S  P  D  Q  T  V  A  S  G  S  E  V  V  L  L  C  T  Y
WM14     TTTGTTTTTCTACAGGGGCACGCTGTGTGACAAAGTAACCCAGAGTTCCCCGGACCAGACGGTGGCGAGTGGCAGTGAGGTGGTACTGCTCTGCACTTAC

          D  T  V  Y  S  N  P  D  L  F  W  Y  R  I  R  P  D  Y  S  F  Q  F  V  F  Y  G  D  N  S  R  S  E  G
WM14     GACACTGTATATTCAAATCCAGATTTATTCTGGTACCGGATAAGGCCAGATTATTCCTTTCAGTTTGTCTTTTATGGGGATAACAGCAGATCAGAAGGTG

         A  D  F  T  Q  G  R  F  S  V  K  H  I  L  T  Q  K  A  F  H  L  V  I  S  P  V  R  T  E  D  S  A  T  Y
WM14     CAGATTTTACTCAAGGACGGTTTTCTGTGAAACACATTCTGACCCAGAAAGCCTTTCACTTGGTGATCTCTCCAGTAAGGACTGAAGACAGTGCCACTTA
                             N    Dδ1     N     Dδ2        N                                 Jδ1
          Y  C  A  F         E     P  T     P     L     V  R  P  F  E      Y  T  D  K  L  I  F  G  K  G  T
WM14     CTACTGTGCCTTT      GAA    CCTAC   TCCT   CTGG   TACGACCGTTCGAAT    ACACCGATAAACTCATCTTTGGAAAAGGAACCC
                                                           Cδ
         R  V  T  V  E  P    R  S  Q  P  H  T  K  P  S  V  F  V  M  K  N  G  T  N  V  A  C  L  V  K  E  F
WM14     GTGTGACTGTGGAACCAA  GAAGTCAGCCTCATACCAAACCATCCGTTTTTGTCATGAAAAATGGAACAAATGTCGCTTGTCTGGTGAAGGAATTC


C

HUMAN V1   QKVTQAQSSVSMPVRKAVTLNCLYETSWWS YYIFWYKQLP  SKEMIFLIRQGSDEQNA KSGRYSVNFKKAAKSVA LTISALQLEDSAKYFCALGE
HUMAN V2   IELVPEHQT-PVSIGVPA--R-SMKGEAIGN---N--RKTQ  GNTIT-IY-EKDIYGPGF- DNFQGDI DI--NL-V-K-L-PSER-EGS-Y--CD
HUMAN V3   D----SSPDQTVASGSE-V-L-T-D- VY-NPDL---RIR-DY-FQFV-YGDNSRS-GADFTQ--F--KHILTQ-AFH -V--PVRT----T-Y--F


HUMAN V1   QKVTQAQSSVSMPVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSD EQNAKSGRYSVNFKKAAKSVALTISALQLEDSAKYFCALGE
MOUSE V6   A-----V--TG-SQWGE ---H-S----EYF-V-L-----F-G--V---Y-T-FTD--QRNS----V-Q-SL--IS-V---S-P---GT-----S


HUMAN V3   DKVTQSSPDQTVASGSEVVLLCTYDTVYSNPDLFWYRIRPDYSFQFVFYGDNSRSEGADFTQGRFSVKHILTQKAFHLVISPVRTEDSATYYCAF
MOUSE V5   ITL----T-------T-AT-----NASPD--------K---R----IL-R-DTS-HD---V--------SKANRT--------SL---------S
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FIGURE 3. Nucleotide and deduced amino acid sequences of $V_{\delta}2$ and $V_{\delta}3$ cDNA clones. (*A*) Sequences of $V_{\delta}2$ cDNAs from cell lines LB117, LB210, and LB207 are presented from near the Eco RI linker at the 5' end of each clone to the Eco RI site situated 77 bp into the C_{δ} segment. Putative contributions of N and D segments within the junctional region are noted. (*B*) Sequence of a $V_{\delta}3$ cDNA from WM14. (*C*) Homologies among the deduced amino acid sequences of human and murine V_{δ} segments. Mouse $V_{\delta}5$ and $V_{\delta}6$ sequences are from reference 22. Dashes denote identities and blanks denote gaps introduced to maximize the alignment. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00792.

blots (Fig. 1, *C* and *D*) and Southern blots (Fig. 4, *A* and *B*). All $V_\delta 1^-$ cell lines expressed either $V_\delta 2$ or $V_\delta 3$ transcripts, although one cell line, LB210, expressed both. The $V_\delta 2$ gene segment was found on a 3.7-kb germline Xba I fragment that was rearranged either to a 4.2-kb fragment (corresponding to rearrangement to $J_\delta 1$) or to a 3.4-kb fragment (corresponding to rearrangement to $J_\delta 2$ or $J_\delta 3$). The $V_\delta 3$ gene segment was found on a 9.4-kb germline Xba I fragment that was rearranged to a 1.9-kb fragment (corresponding to rearrangement to $J_\delta 1$) in two cell lines. As expected from the analysis of Northern blots, LB210 displayed rearrangements of both $V_\delta 2$ and $V_\delta 3$. An additional $V_\delta 3$ rearrangement of ~1.0 kb, not detected by $J_\delta 1$ and $J_\delta 3$ probes (not shown), may reflect rearrangement of $V_\delta 3$ to D_δ. Such V-D rearrangements have been documented for murine TCR-δ (21).

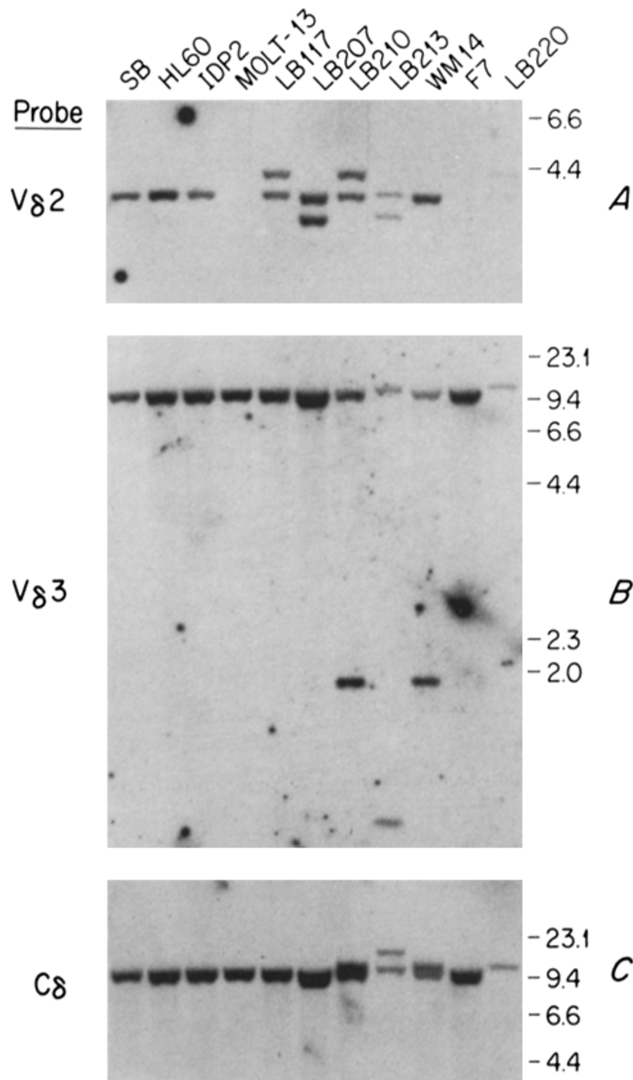

FIGURE 4. Blot hybridization analysis of $V_\delta 2$ and $V_\delta 3$ rearrangements. The blot carrying Xba I-digested DNA samples used in Fig. 2 was serially reprobed with ^{32}P-labeled $V_\delta 2$ (*A*), $V_\delta 3$ (*B*), and C_δ (*C*) DNA fragments.

To determine which of the two rearrangements ($V_δ2$ or $V_δ3$ to $J_δ1$) in LB210 was productive, and which of the $J_δ$ segments ($J_δ2$ or $J_δ3$) was rearranged to $V_δ2$ in LB207, a cDNA library generated from RNA of both cell lines was screened with a $V_δ2$ cDNA probe, as well as with $J_δ1$- and $J_δ3$-specific oligonucleotide probes. Two cDNA clones that used the $V_δ2$ segment along with either $J_δ1$ (LB210) or $J_δ3$ (LB207) were characterized. Their nucleotide and deduced amino acid sequences are presented in Fig. 3 *A*. Both cDNA clones maintain an open reading frame across the V-D-D-J junction, indicating that they represent the functional transcripts in the respective cell lines. Thus, in LB210, which displays both $V_δ2$ and $V_δ3$ rearrangements and transcripts, the $V_δ2$ rearrangement is productive. Further, in LB207, and likely in LB213, productive rearrangements of $V_δ2$ to $J_δ3$ have occurred. This is the first evidence for functional utilization of the human $J_δ3$ element.

We assessed $V_γ$ and $J_γ$ segment usage within this panel by analyzing Northern blots with $V_γ1$ family, $V_γ2$, $V_γ3$, and $V_γ4$ probes (Fig. 1, *E, F, G,* and *H*, respectively) and Southern blots of Kpn I–digested genomic DNA with $J_γ1.3/2.3$ and $V_γ2$ probes (Fig. 5, *A* and *B*, respectively; nomenclature according to references 12 and 14). As shown previously by Huck and Lefranc (15), the linkage of human $J_γ$ segments predicts that a $J_γ1.3/2.3$ probe can detect all TCR-γ gene rearrangements in Kpn I digests of human genomic DNA. Additional analysis using a $V_γ2$ probe served to specifically identify $V_γ2$ rearrangements, and further, to distinguish between rearrangements to $V_γ1$ and $V_γ3$, since the germline $V_γ2$ gene segment would be deleted upon rearrangement of $V_γ1$ gene segments, but retained upon rearrangement of $V_γ3$.

All cell lines within the panel displayed two $J_γ$ rearrangements (Fig. 5 *A*). In some cases Northern blots allowed an assessment of which rearrangement was productive, since only one transcript was detected (Fig. 1, *E, F, G,* and *H*). Further, cells that had productively rearranged the $V_γ2$ gene segment could be identified using the mAb anti-$Ti_γA$, which specifically stains the surface of lymphocytes using this V segment (43). All cells that displayed a $V_γ2$ to $J_γ$ rearrangement were found to be $Ti_γA^+$ by surface fluorescence (data not shown). As a result, unambiguous assignment of the productive rearrangement could be made in most instances.

A summary of the $V_γ$ and $V_δ$ transcripts and $J_γ$ and $J_δ$ rearrangements detected in all of the clonal cell lines that we have examined so far is presented in Table I. All of the cell lines studied appear to productively rearrange one of the three characterized human $V_δ$ segments, and either a $V_γ1$ family member or $V_γ2$. A striking finding of this analysis was that all cell lines within the panel that display productive rearrangement of the $V_δ2$ segment also display productive rearrangement of the $V_γ2$ gene segment. This relationship is not reciprocal, in that the $V_γ2$ gene segment is used in conjunction with either $V_δ1$ or $V_δ2$. The $V_γ2$-$V_δ2$ cell lines within the panel use either $J_γ1.2$ ($J_γP$; 12.0-kb rearrangement) or $J_γ1.3/2.3$ (7.5-kb rearrangement) (Fig. 5 and Table I), and either $J_δ1$ or $J_δ3$, indicating no specific restrictions on J segment utilization. Since the panel of cell lines examined is small, any conclusions must be tentative. However, these data may suggest that TCR-δ chains using the $V_δ2$ gene segment may only be correctly assembled and expressed on the cell surface in association with TCR-γ chains using the $V_γ2$ gene segment. Alternatively, the data may reflect either positive selection for this particular receptor, or negative selection for receptors expressing $V_δ2$ in conjunction with other $V_γ$ segments. Fur-

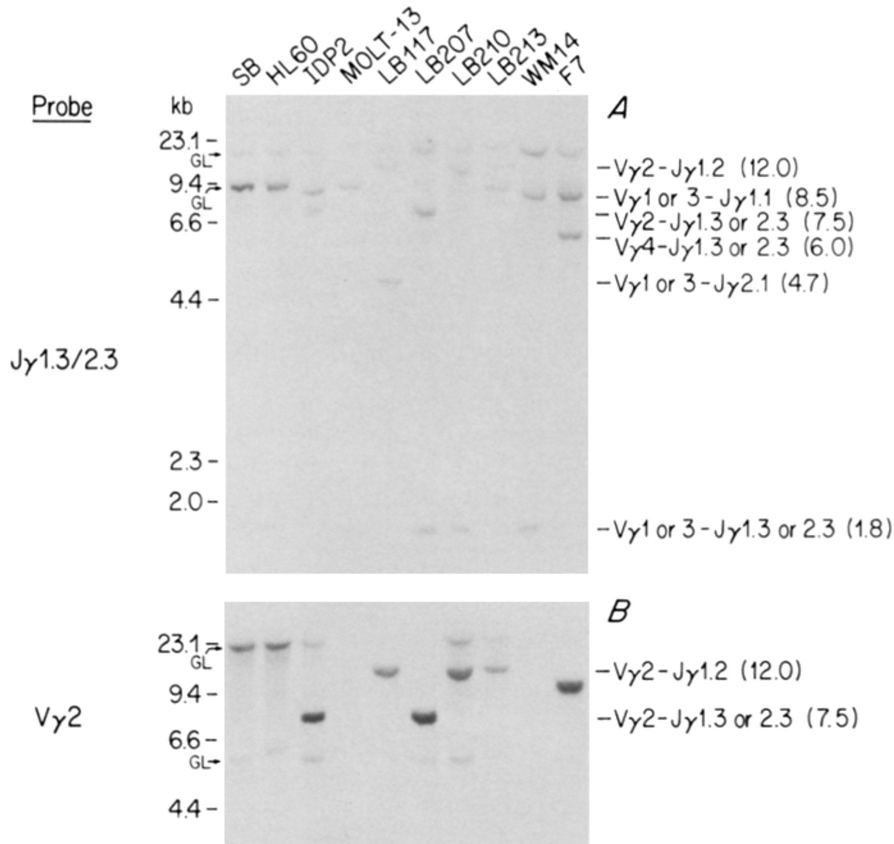

FIGURE 5. Blot hybridization analysis of TCR-γ gene rearrangements. Samples of genomic DNA (5 μg) were digested with Kpn I, subjected to electrophoresis through 0.7% agarose, transferred to Hybond-N membranes, and serially probed with the indicated ^{32}P-labeled DNA fragments. (*A*) The $J_γ1.3/2.3$ probe detects both the $J_γ1$ and $J_γ2$ clusters (germline fragments [GL] of 9 and 16 kb, respectively). The migration of Hind III fragments of phage λ DNA are noted on the left border of the figure, whereas the predicted sizes (in kb) and assignments of rearrangements according to Huck and Lefranc (15) are shown on the right border. (*B*) The $V_γ2$ probe detects two apparent germline fragments in Kpn I digests (GL). The explanation for this is unclear, although the analysis of Xba I digests with this probe reveals that Molt-13, LB117, LB207, and WM14 retain no germline copies of $V_γ2$, a result that correlates with the presence or absence of the larger germline Kpn I fragment (data not shown). It is on this basis that distinction is made between $V_γ1$ and $V_γ3$ rearrangements on the nonproductive chromosome (Table I). The 11-kb fragment in F7 DNA correspond to neither of the $J_γ$ rearrangements in these cells. Whether this represents an unusual rearrangement or a polymorphism is unclear.

ther studies will be necessary to determine the significance of the relationships observed in this panel, and to determine the mechanisms by which these relationships are maintained.

Additional $J_δ1$ Rearrangements in Newborn Thymus DNA. Since there was no evidence for the utilization of additional $V_δ$ segments within this panel of cell lines, we sought to determine whether additional $J_δ$ rearrangements could be detected in Xba I digests of newborn thymus DNA (Fig. 6). A total of seven $J_δ1$ rearrangements

Table I
V_γ and V_δ Transcripts and J_γ and J_δ Rearrangements

	TCR-δ				TCR-γ			
	Rearrangements		Transcripts		Rearrangements		Transcripts	
Cell line	p	n	p	n	p	n	p	n
IDP2	$V_\delta1$-$J_\delta1$	D_δ-$J_\delta1$	$V_\delta1$	-	$V_\gamma2$-$J_\gamma2.3$	$V_\gamma3$-$J_\gamma1.1$	$V_\gamma2$	($V_\gamma3$)
PBL C1	$V_\delta1$-$J_\delta1$	D_δ-$J_\delta1$	$V_\delta1$	-	$V_\gamma2$-$J_\gamma1.3$	ND	$V_\gamma2$	ND
Molt-13	$V_\delta1$-$J_\delta1$	? -$J_\delta1$	$V_\delta1$	-	$V_\gamma1.3$-$J_\gamma2.3$	$V_\gamma1$-$J_\gamma1.1$	$V_\gamma1$	ND
PEER	$V_\delta1$-$J_\delta1$	Deletion	$V_\delta1$	-	$V_\gamma1.8$-$J_\gamma2.3$	$V_\gamma2$-$J_\gamma2.3$	$V_\gamma1$	$V_\gamma2$
LB117	$V_\delta2$-$J_\delta1$	D_δ-$J_\delta1$	$V_\delta2$	-	$V_\gamma2$-$J_\gamma1.2$	$V_\gamma1$-$J_\gamma2.1$	$V_\gamma2$	-
LB207	$V_\delta2$-$J_\delta3$	D_δ-$J_\delta1$	$V_\delta2$	-	$V_\gamma2$-$J_\gamma1.3/2.3$	$V_\gamma1$-$J_\gamma1.3/2.3$	$V_\gamma2$	-
LB210	$V_\delta2$-$J_\delta1$	$V_\delta3$-$J_\delta1$	$V_\delta2$	($V_\delta3$)	$V_\gamma2$-$J_\gamma1.2$	$V_\gamma3$-$J_\gamma1.3/2.3$	$V_\gamma2$	$V_\gamma3$
LB213	$V_\delta2$-$J_\delta3$	-	$V_\delta2$	-	$V_\gamma2$-$J_\gamma1.2$	$V_\gamma3$-$J_\gamma1.1$	$V_\gamma2$	($V_\gamma3$)
LB220	$V_\delta2$-$J_\delta1$	D_δ-$J_\delta1$	$V_\delta2$	-	ND	ND	$V_\gamma2$	$V_\gamma3$
WM14	$V_\delta3$-$J_\delta1$	D_δ-$J_\delta1$	$V_\delta3$	-	[$V_\gamma1$-$J_\gamma1.3/2.3$]	[$V_\gamma1$-$J_\gamma1.1$]	$V_\gamma1$	-
F7	$V_\delta1$-$J_\delta1$	$V_\delta1$-$J_\delta1$	$V_\delta1$	($V_\delta1$)	$V_\gamma1$-$J_\gamma1.1$	$V_\gamma4$-$J_\gamma1.3/2.3$	$V_\gamma1$	-

Nomenclature used to denote TCR-γ gene segments is that of references 12 and 14. The assignments reflect the data obtained in this study as well as in references 17 and 18. The assignment of rearrangements and transcripts as either (p) productive or (n) nonproductive results from a combination of blot hybridization data, cDNA sequences, and staining with mAb anti-TiγA. Unambiguous assignment of the productive rearrangement could be made in each case, except for the TCR-γ rearrangements in WM14, which are bracketed. $V_\gamma1$ family segments are denoted explicitly when the information is available from sequence analysis (e.g., $V_\gamma1.8$), or simply as $V_\gamma1$ when the assignment relies solely on hybridization data that does not distinguish $V_\gamma1$ family segments from one another. Transcripts detected at very low levels are in parentheses.

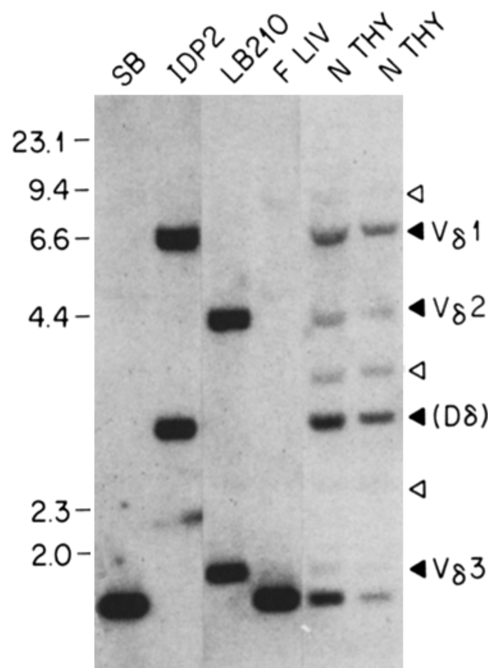

Figure 6. Additional $J_\delta1$ rearrangements in newborn thymus DNA. Samples of genomic DNA (5 μg) were digested with Xba I, subjected to electrophoresis through 0.7% agarose, transferred to Hybond-N, and probed with the ^{32}P-labeled $J_\delta1$ DNA fragment. SB displays two germline $J_\delta1$ alleles, IDP2 displays $J_\delta1$ rearrangements to $V_\delta1$ and to D_δ, and LB210 displays $J_\delta1$ rearrangements to $V_\delta2$ and $V_\delta3$. F LIV represents 20-wk fetal liver DNA, and N THY represents two samples of newborn thymus DNA (4 d and 12 wk). Rearrangements in the thymus samples that are represented within the clonal cell lines are marked by filled arrowheads, whereas those not represented within the clonal cell lines are marked with open arrowheads.

were detected in each of two DNA samples. Those of 6.4, 4.2, and 1.9 kb correspond to rearrangement to $V_\delta 1$, $V_\delta 2$, and $V_\delta 3$, respectively, whereas that of 2.9 kb corresponds to the common rearrangement (thought to be partial D-J or D-D-J) shared by many cell lines. Of the three remaining $J_\delta 1$ rearrangements (8.2, 3.4, and 2.4 kb; Fig. 6, *open triangles*), one might reflect the second partial rearrangement (D-J or D-D-J). Thus, the $J_\delta 1$ probe may detect rearrangements to two or three as yet unidentified V segments. The low frequency of rearrangements detected by the $J_\delta 3$ probe precluded an analysis of rearrangements to the $J_\delta 2$ or $J_\delta 3$ gene segments in total thymocyte populations. However, these data suggest strongly that the number of frequently rearranged human V_δ segments will not be large.

Dispersed Organization of Human V_δ Segments within the TCR-α/δ Locus. Our initial studies of the genomic organization of the TCR-α/δ locus revealed that the $V_\delta 1$ genomic segment was a large distance from the C_δ locus, with one V_α segment ($V_\alpha 13.1$) immediately 5′ of $V_\delta 1$, and at least one V_α segment ($V_\alpha 17.1$) between $V_\delta 1$ and D_δ-J_δ-C_δ (20). These data indicated that V_α and V_δ segments were interspersed within the locus. To determine whether V_δ segments were clustered or dispersed, we determined the locations of the newly identified $V_\delta 2$ and $V_\delta 3$ segments. As seen in Fig. 2 *C*, the F7 cell line, which has rearranged $V_\delta 1$ to $J_\delta 1$ on both chromosomes (Fig. 2, *A* and *B* and reference 20), has deleted both copies of the $V_\delta 2$ gene segment. This places the $V_\delta 2$ gene segment in the region between $V_\delta 1$ and the C_δ locus. However, whereas previous studies have indicated that the $V_\alpha 17.1$ gene segment maps by field inversion gel electrophoresis to the Sal I and Sfi I fragments carrying $V_\delta 1$ (20), $V_\delta 2$ maps to the Sfi I and Sal I fragments carrying D_δ-J_δ-C_δ (data not shown). Thus, $V_\delta 2$ lies between $V_\alpha 17.1$ and D_δ-J_δ-C_δ, and must be within 100 kb 5′ of the latter (Fig. 7).

It is intriguing that like F7, Molt-13 also has deleted both copies of the $V_\delta 2$ gene segment. Molt-13 displays a productive $V_\delta 1$ to $J_\delta 1$ rearrangement on one chromosome, and an unidentified $J_\delta 1$ rearrangement on the other chromosome, with one

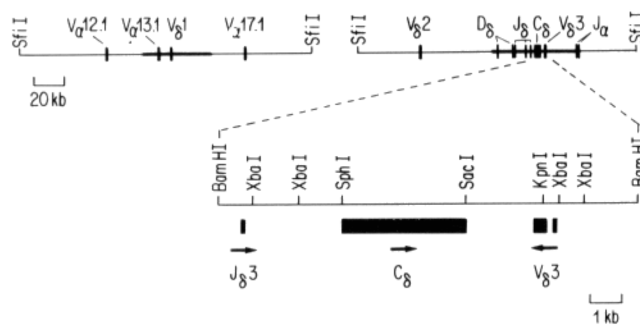

FIGURE 7. Dispersed genomic organization of V_δ gene segments. Sfi I fragments of 190 and 180 kb carrying the $V_\delta 1$ and C_δ segments are shown. The linkage between these fragments has not been established. The order of the gene segments mapping onto these fragments has been established in genomic Southern blotting experiments and by the mapping of cosmid clones (in the regions covered by the bold horizontal lines). However, the precise distances between the segments mapping outside the regions covered by cosmid clones are uncertain. The nomenclature for V_α segments is according to Klein et al. (46). Since we have only attempted to map a limited number of V_α segments, additional unidentified V_α segments may also map to these fragments. A more detailed restriction map of two adjacent subcloned Bam HI–Kpn I fragments that carry the $J_\delta 3$, C_δ, and $V_\delta 3$ segments is also presented. Transcriptional orientations are denoted by arrows. The orientation of the $J_\delta 3$ and $V_\delta 3$ segments with respect to mapped restriction sites has been directly established by nucleotide sequence analysis. The orientation of the C_δ segment has been established elsewhere.

copy of $V_\delta 1$ in the germline configuration (18). This, together with field inversion gel electrophoresis mapping (data not shown), suggests that the nonproductive $J_\delta 1$ rearrangement in Molt-13 involves an unidentified region mapping between $V_\alpha 17.1$ and $V_\delta 2$.

Unlike $V_\delta 2$, the $V_\delta 3$ gene segment is not deleted upon rearrangement of $V_\delta 1$ to $J_\delta 1$ (Fig. 4 *B*), indicating that it does not lie between these gene segments. Further, $V_\delta 3$ rearrangements to $J_\delta 1$ were detected by a C_δ probe (Fig. 4, *B* and *C*), indicating that $V_\delta 3$ lies extremely close to C_δ. The analysis of cosmid clones spanning the C_δ region demonstrated that $V_\delta 3$ lies in an inverted orientation, roughly 2-3 kb 3' of the C_δ segment (Fig. 7). After subcloning and fine mapping of the Kpn I-Bam HI fragments spanning this region, the nucleotide sequence of the germline $V_\delta 3$ segment was determined. This analysis revealed a structure typical of other TCR V segments, including separate exons encoding the leader peptide and the main body of the V segment, and heptamer and nonamer recombination signals flanking the 3' end of the second exon, thereby establishing its boundary (Fig. 8). The location and orientation of the $V_\delta 3$ segment relative to C_δ is analogous to that described for murine $V_\beta 14$ relative to $C_\beta 2$ (44), and implies that rearrangement of $V_\delta 3$ occurs by inversion of D_δ-J_δ-C_δ. These data provide further support for the notion that a limited number of human V_δ segments are dispersed within the human TCR-α/δ locus.

Discussion

The present study represents an extension of previous work carried out by ourselves and others detailing the diversity and organization of human TCR-δ gene segments. Previous studies have identified two D_δ segments, three J_δ segments, and one C_δ segment, nested within the TCR-α locus, and located just upstream of the J_α region (20, 23-26). An estimated 50-100 V_α segments are situated in the region 5' of the D_δ, J_δ, C_δ, J_α, and C_α segments (2, 26). Despite the large number of V segments available for recombination, all productive TCR-δ gene rearrangements in previously examined TCR-γ/δ cell lines involve a single V segment, denoted $V_\delta 1$ (16-18). This V segment is distinct from previously characterized V_α segments, yet is situated among V_α segments, at least 180 kb 5' of $J_\delta 1$ (20). These observations

$V_\delta 3$

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TCCTCTTGTTTTCCTCCCGTCTTCTTTCATTAGAACCGTCAGATAGTCTCTGAGATCTTAGGAACCCTGTTTTGTTGTAT

                         M  I  L  T  V  G  F  S  F  L  F  F
ATCCTCTGCCCTGATATGAAGAAAAGATGATTCTTACTGTGGGCTTTAGCTTTTTGTTTTTCTGTAAGTAGTTTCATTTG

TTTACATTCCACTTGCTCTATAACCCCATTTCTCCTTCTCTGACTCTTGAAGGCTTTTTCTCTTGCACTCTTGGTTTTTG

GTTATTTGCTGCCTTTGGCTTTATTTCTGTGTCCTTCTCTTGCCTCTGCTCATTGAGTTCTTCTGGGCTAGGCATGTGCT

                                                         Y  R  G  T  L  C  D  K
GTACTCACTGTCTGGGGGGTGCAGAATTCACTATTTCCTCATTGTCTTTTTCCCAGACAGGGGCACGCTGTGTGACAAAG

V  T  Q  S  S  P  D  Q  T  V  A  S  G  S  E  V  V  L  L  C  T  Y  D  T  V  Y  S
TAACCCAGAGTTCCCCGGACCAGACGGTGGCGAGTGGCAGTGAGGTGGTACTGCTCTGCACTTACGACACTGTATATTCA

 N  P  D  L  F  W  Y  R  I  R  P  D  Y  S  F  Q  F  V  F  Y  G  D  N  S  R  S  E
AATCCAGATTTATTCTGGTACCGGATAAGGCCAGATTATTCCTTTCAGTTTGTCTTTTATGGGGATAACAGCAGATCAGA

  G  A  D  F  T  Q  G  R  F  S  V  K  H  I  L  T  Q  K  A  F  H  L  V  I  S  P
AGGTGCAGATTTTACTCAAGGACGGTTTTCTGTGAAACACATTCTGACCCAGAAAGCCTTTCACTTGGTGATCTCTCCAG

V  R  T  E  D  S  A  T  Y  Y  C  A  F
TAAGGACTGAAGACAGTGCCACTTACTACTGTGCCTTTAGCACTATGATGCAGGTGCCCAGGAAGTCATAACACAAACTC
```

FIGURE 8. Nucleotide and deduced amino acid sequence of the $V_\delta 3$ genomic segment. Splice donor and acceptor sequences, as well as hepamer and nonamer recombination signals, are underlined. These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00792.

suggested a limited repertoire of germline TCR-δ gene segments, and a highly controlled rearrangement process that is able to segregate TCR-δ and TCR-α recombinational events and repertoires, despite the interspersion of TCR-δ and TCR-α gene segments.

Our analysis of a larger panel of TCR-γ/δ cell lines has now allowed the identification of two additional $V_δ$ segments, termed Vδ2 and Vδ3. Like Vδ1, these $V_δ$ segments are distinct from previously characterized $V_α$ segments. Blot hybridization experiments indicate that Vδ2 lies within 100 kb 5' of the $C_δ$ region, and analysis of cosmid clones reveals that Vδ3 lies ~2–3 kb 3' of $C_δ$. These results clearly demonstrate that $V_δ$ segments are not present as a discrete cluster, but rather are dispersed within the locus. It follows that the segregation of $V_α$ and $V_δ$ rearrangements may be mediated by local signals marking particular V segments, rather than long range signals marking large segments of the locus. Such signals might include unique promoter or other elements that could help to modulate the accessibility of particular V segments to recombinases at a specific time in development (45), but probably do not include the heptamer and nonamer recombination signals flanking the V segments, since they are indistinguishable.

Of the three human $V_δ$ segments already characterized, two display quite high amino acid sequence identity with particular murine $V_δ$ segments. Identity is 58% in a comparison of human Vδ1 with a murine Vδ6 family member, and 66% in a comparison of human Vδ3 with murine Vδ5. Conservation extends to genomic organization as well, since murine Vδ5, like human Vδ3, lies 3' to the $C_δ$ segment, in an inverted orientation (A. Korman, personal communication), whereas murine Vδ6, like human Vδ1, lies at some distance 5' to $C_δ$. This conservation strengthens the emerging close relationship between the global organizations of the human and murine TCR-α/δ loci.

The analysis of Jδ1 rearrangements in newborn thymus DNA supports the likelihood that there may be two or three additional $V_δ$ segments utilized within this polyclonal population that are not represented within the clonal cell lines that we have examined to date. Since the rearrangements in question occur at low frequencies within the thymus, their characterization probably awaits the identification of clonal cell lines displaying these rearrangements. It is also possible that distinct and novel $V_δ$ segments may be rearranged at a selective time in thymic development and/or in TCR-γ/δ cells populating distinct sites in the periphery and hence might not be represented in the polyclonal samples we have analyzed.

It is important to note that the vast majority of Jδ1 rearrangements detected in newborn thymocyte DNA must occur in cells that do not express functional TCR-γ/δ on their surfaces, since only 0.5–1.0% of the thymocytes in these samples stained with the mAb anti-TCR-δ1 (V. Groh, personal communication). Based upon the intensity of the rearrangements relative to control single copy examples in clonal cell lines, we assume that the fraction of thymocytes displaying Jδ1 rearrangements is much higher. This would argue that the detection of a limited number of Jδ1 rearrangements could not reflect selection for the expression of functional receptors, but rather must reflect specificity in the rearrangement process per se.

Current information indicates that the TCR-γ/δ V segment repertoires are both limited and of similar size in man and mouse. In man there are at most seven functional $V_γ$ segments (in four families), and at least three functional $V_δ$ segments. In

mouse there are six functional V_γ segments (in five families) and at least nine functional $V\delta$ segments (in six families) (7, 22). These numbers are significantly lower than those estimated for TCR-α/β, which range from 30 to 100 for each V segment (2, 3). Based upon our limited panel analysis, it may be inappropriate to assume combinatorial association of V_γ and V_δ segments, since five of five examples of TCR-γ/δ cell lines using the $V_\delta 2$ segment also used the $V_\gamma 2$ segment. Whether this might reflect constraints imposed on chain pairing or on receptor specificity is unclear, but it nevertheless suggests that the total number of V_γ-V_δ pairs may be limited. Such data could be interpreted to indicate that TCR-γ/δ might interact with a discrete set of putative restricting elements or antigen-presenting molecules. The resolution of this issue clearly awaits the derivation and study of antigen-specific TCR-γ/δ T cell lines and clones.

Summary

Previous studies of the human TCR-δ gene identified a single commonly used V_δ segment, denoted $V_\delta 1$. To better understand the extent of the human TCR-δ V gene repertoire, TCR-δ transcripts and gene rearrangements were examined in a new panel of cloned human TCR-γ/δ lymphocytes. Through this analysis we identified and determined the structures of two new V_δ segments, denoted $V_\delta 2$ and $V_\delta 3$. These V_δ segments are different from previously characterized V_α segments, supporting the notion that the human V_δ and V_α repertoires are distinct. Examination of V_γ gene segment usage in these cells reveals that the $V_\delta 2$ gene segment is used in conjunction with the $V_\gamma 2$ gene segment. Blot hybridization indicates that the $V_\delta 2$ gene segment lies between $V_\delta 1$ and D_δ-J_δ-C_δ, and within 100 kb of the latter. Analysis of genomic clones indicates that the $V_\delta 3$ gene segment lies in an inverted orientation, ~2 kb 3′ of C_δ. This implies that rearrangement of $V_\delta 3$ to D_δ-J_δ-C_δ occurs by inversion. Together with previous mapping studies, these results indicate that human V_δ segments are dispersed, rather than clustered, within the TCR-α/δ locus. The analysis of rearrangements in polyclonal thymocyte DNA suggests that there may be a limited number of additional V_δ gene segments yet to be characterized.

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Note added in proof: We have identified two additional T cell clones that apparently express a $V_\gamma 2$-$V_\delta 2$ receptor.

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References

1. Marrack, P., and J. Kappler. 1987. The T cell receptor. *Science (Wash. DC).* 238:1073.
2. Toyonaga, B., and T. W. Mak. 1987. Genes of the T-cell antigen receptor in normal and malignant T cells. *Annu. Rev. Immunol.* 5:585.
3. Kronenberg, M., G. Siu, L. E. Hood, and N. Shastri. 1986. The molecular genetics of the T-cell antigen receptor and T-cell antigen recognition. *Annu. Rev. Immunol.* 4:529.

4. Brenner, M. B., J. McLean, D. P. Dialynas, J. L. Strominger, J. S. Smith, F. L. Owen, J. G. Seidman, S. Ip, F. Rosen, and M. S. Krangel. 1986. Identification of a putative second T-cell receptor. *Nature (Lond.).* 322:145.
5. Bank, I., R. A. DePinho, M. B. Brenner, J. Cassimeris, F. W. Alt, and L. Chess. 1986. A functional T3 molecule associated with a novel heterodimer on the surface of immature human thymocytes. *Nature (Lond.).* 322:179.
6. Lew, A. M., D. M. Pardoll, W. L. Maloy, B. J. Fowlkes, A. Kruisbeek, S.-F. Cheng, R. N. Germain, J. A. Bluestone, R. H. Schwartz, and J. E. Coligan. 1986. Characterization of T-cell receptor gamma chain expression in a subset of murine thymocytes. *Science (Wash. DC).* 234:1401.
7. Brenner, M. B., J. L. Strominger, and M. S. Krangel. 1988. The $\gamma\delta$ T cell receptor. *Adv. Immunol.* 43:133.
8. Koning, F., G. Stingl, W. M. Yokoyama, H. Yamada, W. L. Maloy, E. Tschachler, E. M. Shevach, and J. E. Coligan. 1987. Identification of a T3-associated $\gamma\delta$ T cell receptor on Thy-1$^+$ dendritic epidermal cell lines. *Science (Wash. DC).* 236:834.
9. Goodman, T., and L. Lefrancois. 1988. Expression of the γ-δ T-cell receptor on intestinal CD8$^+$ intraepithelial lymphocytes. *Nature (Lond.).* 333:855.
10. Matis, L., R. Cron, and J. A. Bluestone. 1987. Major histocompatibility complex-linked specificity of $\gamma\delta$ receptor-bearing T lymphocytes. *Nature (Lond.).* 330:262.
11. Forster, A., S. Huck, N. Ghanem, M.-P. Lefranc, and T. H. Rabbitts. 1987. New subgroups in the human T cell rearranging V_γ gene locus. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:1945.
12. Strauss, W. M., T. Quertermous, and J. G. Seidman. 1987. Measuring the human T cell receptor γ-chain locus. *Science (Wash. DC).* 237:1237.
13. Huck, S., P. Dariavich, and M.-P. Lefranc. 1988. Variable region genes in the human T-cell rearranging gamma (TRG) locus: V-J junction and homology with the mouse genes. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:719.
14. Quertermous, T., W. M. Strauss, J. J. M. van Dongen, and J. G. Seidman. 1987. Human T cell γ chain joining regions and T cell development. *J. Immunol.* 138:2687.
15. Huck, S., and M.-P. Lefranc. 1987. Rearrangements to the JP1, JP and JP2 segments in the human T-cell rearranging gamma gene (TRGγ) locus. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 224:291.
16. Hata, S., M. B. Brenner, and M. S. Krangel. 1987. Identification of putative human T cell receptor δ complementary DNA clones. *Science (Wash. DC).* 238:687.
17. Loh, E. Y., L. L. Lanier, C. W. Turck, D. R. Littman, M. M. Davis, Y.-H. Chien, and A. Weiss. 1987. Identification and sequence of a fourth human T cell antigen receptor chain. *Nature (Lond.).* 330:569.
18. Hata, S., K. Satyanarayana, P. Devlin, H. Band, J. McLean, J. L. Strominger, M. B. Brenner, and M. S. Krangel. 1988. Extensive junctional diversity of rearranged human T cell receptor δ genes. *Science (Wash. DC).* 240:1541.
19. Boehm, T., L. Buluwela, D. Williams, L. White, and T. H. Rabbitts. 1988. A cluster of chromosome 11p13 translocations found via distinct D-D and D-D-J rearrangements of the human T cell receptor δ chain gene. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:2011.
20. Satyanarayana, K., S. Hata, P. Devlin, M. G. Roncarolo, J. E. De Vries, H. Spits, J. L. Strominger, and M. S. Krangel. 1988. Genomic organization of the human T cell receptor α/δ locus. *Proc. Natl. Acad. Sci. USA.* In press.
21. Chien, Y.-H., M. Iwashima, D. A. Wettstein, K. B. Kaplan, J. F. Elliott, W. Born, and M. M. Davis. 1987. T-cell receptor δ gene rearrangements in early thymocytes. *Nature (Lond.).* 330:722.
22. Elliott, J. F., E. P. Rock, P. A. Patten, M. M. Davis, and Y.-H. Chien. 1988. The adult T-cell receptor δ-chain is diverse and distinct from that of fetal thymocytes. *Nature (Lond.).* 331:627.

23. Takihara, Y., D. Tkachuk, E. Michalopoulos, E. Champagne, J. Reimann, M. Minden, and T. W. Mak. 1988. Sequence and organization of the diversity, joining, and constant region genes of the human T-cell δ-chain locus. *Proc. Natl. Acad. Sci. USA.* 85:6097.
24. Boehm, T., R. Baer, I. Lavenir, A. Forster, J. J. Waters, E. Nacheva, and T. H. Rabbitts. 1988. The mechanism of chromosomal translocation t(11;14) involving the T-cell receptor Cδ locus on human chromosome 14q11 and a transcribed region of chromosome 11p15. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:385.
25. Isobe, M., G. Russo, F. G. Haluska, and C. M. Croce. 1988. Cloning of the gene encoding the δ subunit of the human T-cell receptor reveals its physical organization within the α-subunit locus and its involvement in chromosome translocations in T-cell malignancy. *Proc. Natl. Acad. Sci. USA.* 85:3933.
26. Griesser, H., E. Champagne, D. Tkachuk, Y. Takihara, M. Lalande, E. Baillie, M. Minden, and T. W. Mak. 1988. The human T cell receptor α-δ locus: a physical map of the variable, joining and constant region genes. *Eur. J. Immunol.* 18:641.
27. Hochstenbach, F., C. Parker, J. McLean, V. Gieselmann, H. Band, I. Bank, L. Chess, H. Spits, J. L. Strominger, J. G. Seidman, and M. B. Brenner. 1988. Characterization of a third form of the human T cell receptor γ/δ. *J. Exp. Med.* 168:761.
28. Alarcon, B., J. De Vries, C. Pettey, A. Boylston, H. Yssel, C. Terhorst, and H. Spits. 1987. The T-cell receptor γ chain-CD3 complex: Implication in the cytotoxic activity of a $CD3^+CD4^-CD8^-$ human natural killer clone. *Proc. Natl. Acad. Sci. USA.* 84:3861.
29. Yssel, H., J. E. De Vries, M. Koken, W. V. Van Blitterswijk, and H. Spits. 1984. A serum free medium for the generation and propagation of functional human cytotoxic and helper T cell clones. *J. Immunol. Methods.* 72:219.
30. Krangel, M. S., H. Band, S. Hata, J. McLean, and M. B. Brenner. 1987. Structurally divergent T cell receptor γ proteins encoded by distinct C_γ genes. *Science (Wash. DC).* 237:64.
31. Feinberg, A., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6.
32. Auffray, C., and F. Rougeon. 1980. Purification of mouse immunoglobulin heavy chain messenger RNAs from total myeloma tumor RNA. *Eur. J. Biochem.* 107:303.
33. Wigler, M., R. Sweet, G. K. Sim, B. Wold, A. Pellicer, E. Lacey, T. Maniatis, S. Silverstein, and R. Axel. 1979. Transformation of mammalian cells with genes from procaryotes and eukaryotes. *Cell.* 16:777.
34. Lehrach, H., D. Diamond, J. M. Wozney, and H. Boedtker. 1977. RNA molecular weight determination by gel electrophoresis under denaturing conditions, a critical reexamination. *Biochemistry.* 16:4743.
35. Huynh, T. V., R. A. Young, and R. W. Davis. 1985. Construction and screening cDNA libraries in λgt10 and λgt11. *In* DNA Cloning, A Practical Approach, Vol I. D. M. Glover, editor. IRL Press Limited, Oxford. 49–78.
36. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463.
37. Biggin, M. D., T. J. Gibson, and G. F. Hong. 1983. Buffer gradient gels and [35]S label as an aid to rapid DNA sequence determination. *Proc. Natl. Acad. Sci. USA.* 80:3963.
38. Tabor, S., and C. C. Richardson. 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. USA.* 84:4767.
39. Chen, E. Y., and P. H. Seeburg. 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. *DNA (NY).* 4:165.
40. Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. *Gene.* 28:351.
41. Lai, E., P. Concannon, and L. Hood. 1988. Conserved organization of the human and murine T-cell receptor β-gene families. *Nature (Lond.).* 331:543.

42. Pelkonen, J., A. Traunecker, and K. Karjalainen. 1987. A new mouse TCR V_γ gene that shows remarkable evolutionary conservation. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:1941.
43. Treibel, F., F. Faure, M. Graziana, S. Jitsukawa, M.-P. Lefranc, and T. Hercend. 1988. A unique V-J-C-rearranged gene encodes a γ protein expressed on the majority of $CD3^+$ T cell receptor-α/β^- circulating lymphocytes. *J. Exp. Med.* 167:694.
44. Malissen, M., C. McCoy, D. Blanc, J. Trucy, C. Devaux, A.-M. Schmitt-Verhulst, F. Fitch, L. Hood, and B. Malissen. 1986. Direct evidence for chromosomal inversion during T-cell receptor β gene rearrangements. *Nature (Lond.).* 319:28.
45. Alt, F., T. K. Blackwell, and G. D. Yancopoulos. 1987. Development of the primary antibody repertoire. *Science (Wash. DC).* 238:1079.
46. Klein, M. H., P. Concannon, M. Everett, L. D. H. Kim, T. Hunkapiller, and L. Hood. 1987. Diversity and structure of human T-cell receptor α-chain variable region genes. *Proc. Natl. Acad. Sci. USA.* 84:6884.