Brief Definitive Report

STRUCTURE OF THE γ/δ T CELL RECEPTOR OF A HUMAN THYMOCYTE CLONE

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Most murine and human T cells express an antigen receptor (TCR) composed of a disulfide-linked heterodimer of two Ig-like proteins, termed α and β, that is associated with an invariant complex of proteins termed CD3 (1). Recently, a second TCR was found on the surface of a small percentage of thymocytes and peripheral T cells (reviewed in reference 1). This TCR consists of two chains termed γ and δ, which can be either disulfide linked or noncovalently associated (2). Like the TCR-α/β, the TCR-γ/δ is expressed on the cell membrane in association with the CD3 complex (2–5). The biological functions and ligands for the TCR-γ/δ are unknown. We previously described a $CD3^+$, IL-2-dependent normal human thymocyte clone, termed CII, that expressed a CD3-associated heterodimer composed of a 40-kD γ chain noncovalently associated with a 38-kD protein presumably representing a δ chain (3). The putative CII γ chain is smaller than previously characterized, non-covalently linked γ proteins (2–4). To define the nature of the CII TCR, we have characterized cDNA and genomic clones from CII that encode functional TCR γ and δ chains.

Materials and Methods

Cell Culture. The CII clone was isolated from a normal human thymus by culturing $CD4^-$, $CD8^-$ thymocytes in the presence of PHA, IL-2, and lymphoblastoid feeder cells, as described in detail elsewhere (3).

Nucleic Acid Analysis. Nucleic acids were prepared and analyzed by blotting/hybridization methods as described previously; genomic and cDNA libraries were prepared as previously described (6).

Probes. The J_γ probe pH60 was kindly provided by T. H. Rabbitts (MRC, Cambridge, UK). TCR-γ and -δ (CXHYO; reference 7) cDNA probes have been described. A 21-mer 5'-AATGTCGCTTGTCTGGTGAAG-3' was synthesized to the sense strand of nucleotides 427–447 of a composite TCR-δ sequence (8) and used to isolate the δ cDNA.

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Result and Discussion

Expression and Rearrangement of TCR Genes in CII. The CII line does not produce detectable levels of complete (V[D]J-C) TCR-α and -β mRNA species or proteins, but does produce sequences of appropriate size to represent complete TCR-γ and -δ mRNA (3, 7). To further characterize the CII TCR, we assayed for expression of surface TCR components by cytoflourography. CII expresses surface CD3 and TCR δ chains, but does not express determinants recognized by an antibody to consensus regions of the TCR-α,β complex (data not shown). Thus, these findings confirm that CII expresses a TCR-γ/δ.

To characterize potential rearrangements of TCR-γ genes, CII genomic DNA was digested with Bam HI and assayed by Southern blotting for hybridization to a $J_\gamma 1$ probe (pH60). The 5′ region of the $C_\gamma 1$ and $C_\gamma 2$ genes, respectively, are contained on germline pH60-hybridizing Bam HI fragments of ~18 and ~14 kb (Fig. 1 *A*, lane *BA*). The 14-kb fragment is retained in CII DNA, but both copies of the 18-kb fragment have been deleted, accompanied by the appearance of a novel pH60-hybridizing 20-kb fragment, suggesting that CII has rearranged or deleted *$C_\gamma 1$* on both alleles, and maintained the germline configuration for $C_\gamma 2$ on at least one allele (Fig. 1 *A*). To characterize rearrangements of the TCR-δ locus, Eco RI-digested genomic DNA was assayed for hybridization to a *$J_\delta 1/C_\delta$*-specific cDNA probe (7).

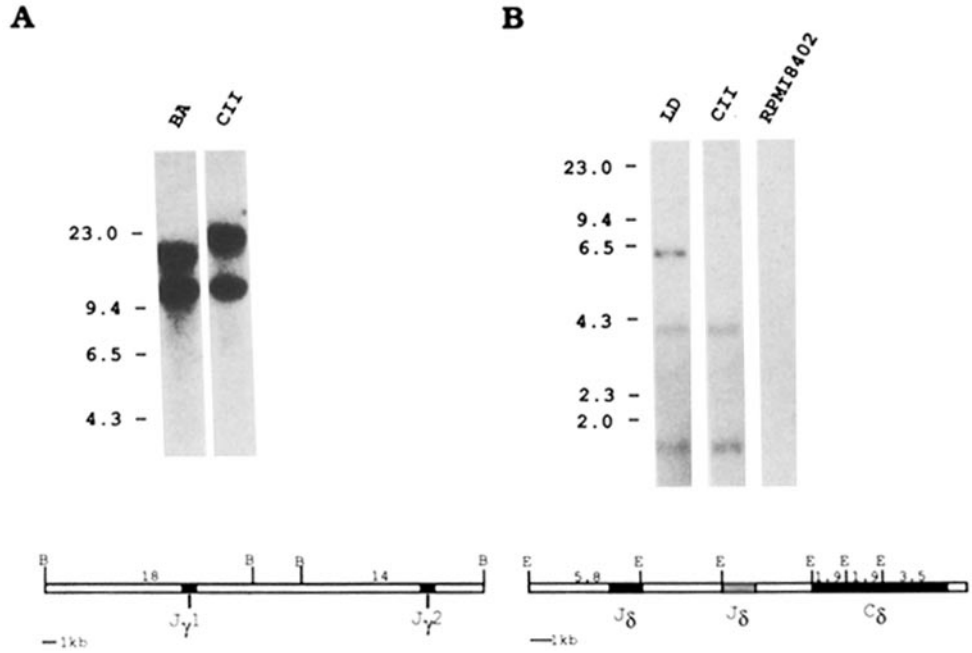

FIGURE 1. Rearrangement of γ and δ genes in CII. (*A*) 10 μg of genomic DNA from human B cell lines (*BA* and *LD*), human T cell leukemia line (*RPMI8402*), or *CII* was assayed by genomic blotting procedures for hybridization to the $J_\gamma 1$- and $J_\gamma 2$-hybridizing probe pH60. The map of the genomic J_γ region, as previously reported (8), is indicated below. (*B*) 10 μg genomic DNA was digested with Eco R1 and assayed for hybridization to ^{32}P-labeled J_δ-C_δ-containing cDNA probe. The map of the J_δ-C_δ region is indicated below as previously reported (10). Regions hybridizing to the probe are indicated in black.

B cell DNA contained three Eco R1 fragments of ~6, ~3.5, and ~1.9 kb that hybridized to the δ-specific probe (Fig. 1 *B*; lane *LD*). In CII DNA, the 6-kb fragment was deleted while the 3.5 and 1.9-kb fragments were present (Fig. 1 *B*). The disappearance of the 6-kb band could be due to rearrangement or deletion of J_δ, as the probe has 5' but not 3' flanking germline sequences of J_δ (Fig. 1 *B*, *bottom*; 7). Thus, both the C_γ and C_δ loci are rearranged in CII.

Isolation of the TCR-γ cDNA and Genomic Clones. To characterize the TCR-γ RNA transcripts from CII cells, we isolated C_γ-hybridizing cDNA clones from a CII cDNA library. Two distinct $V_\gamma JC_\gamma$-containing cDNA clones, neither of which contained the entire V region, were identified. One contained a V sequence joined via $J_\gamma 2$ to the $C_\gamma 2$ region; the V and C sequences were in the same translational reading frame demonstrating that this cDNA represented the productively rearranged γ allele (Fig. 2 *A*, *GC12*). To obtain the complete V_γ region of this transcript, a Hind III fragment that contained this rearrangement was isolated from a CII genomic library (Fig. 2 *A*, *GH3*). The composite nucleotide sequences of the cDNA and genomic clones demonstrated that this V segment derived from the V_γI family (Fig. 2 *A*, reference 9). Another cDNA sequence contained a V_γIII V region joined via $J_\gamma P1$ (9) to $C_\gamma 1$ but in a different translational reading frame, indicating that it represented the nonproductively rearranged γ allele (Fig. 2 *B*). Possible N regions were present in the VJ junctions of both cDNA sequences (9; Fig. 2, *A* and *B*).

Although the two constant regions in the human TCR-γ locus are homologous, the number of copies of the exon 2 domain and several base differences within it make $C_\gamma 1$ and $C_\gamma 2$ structurally distinct (4). While the invariant single copy of exon 2 in $C_\gamma 1$ encodes a cysteine residue that covalently links the TCR-γ/δ chains (used in the nonproductive CII rearrangment; Fig. 2), the exon 2 domains used in $C_\gamma 2$ do not have this cysteine residue and therefore specify a non-disulfide-linked form of the receptor (4). The use of the $C_\gamma 2$ constant region in the productive γ rearrangement is consistent with the observation that the γ and δ chains of the CII TCR are not covalently linked (2, 3). Furthermore, the $C_\gamma 2$ constant region expressed by the CII line derives from the polymorphic form that contains two 48-bp copies of exon 2 (denoted CII' and CII" in Fig. 2 *A*). This finding accounts for the observation that the TCR-γ peptide expressed by CII is smaller than the γ peptide expressed by Peer T cell lymphoma line, which uses the three-domain configuration of the $C_\gamma 2$ gene, making the latter larger by 16 amino acids and providing an additional glycosylation site (4). Although the two-domain form of the C_γ polymorphism was found to occur more frequently in the population (10), CII is the first normal T cell line reported to express a γ chain derived from this allele. In parallel, others have found that the Molt-13 T cell leukemia expresses this $C_\gamma 2$ allele (11).

Isolation of cDNA-encoding TCR δ Chain. To further characterize the CII TCR δ chain, we isolated C_δ-hybridizing cDNA clones from a CII cDNA library. The nucleotide sequence of one such clone (D105) reveals a V_δ gene segment productively joined to J_δ (Fig. 3); this V_δ segment is identical to a previously characterized single copy V gene (8, 12). The V and J segments of D105 are joined by a 20-bp region that cannot be attributed to known TCR-δ V or J segments (7, Fig. 3). These extra nucleotides could potentially accomodate a D segment, perhaps more than one, as in murine TCR-δ rearrangements (13) (Fig. 3). The TCR-δ mRNA expressed by CII uses the same V and J regions expressed by a peripheral blood-derived T

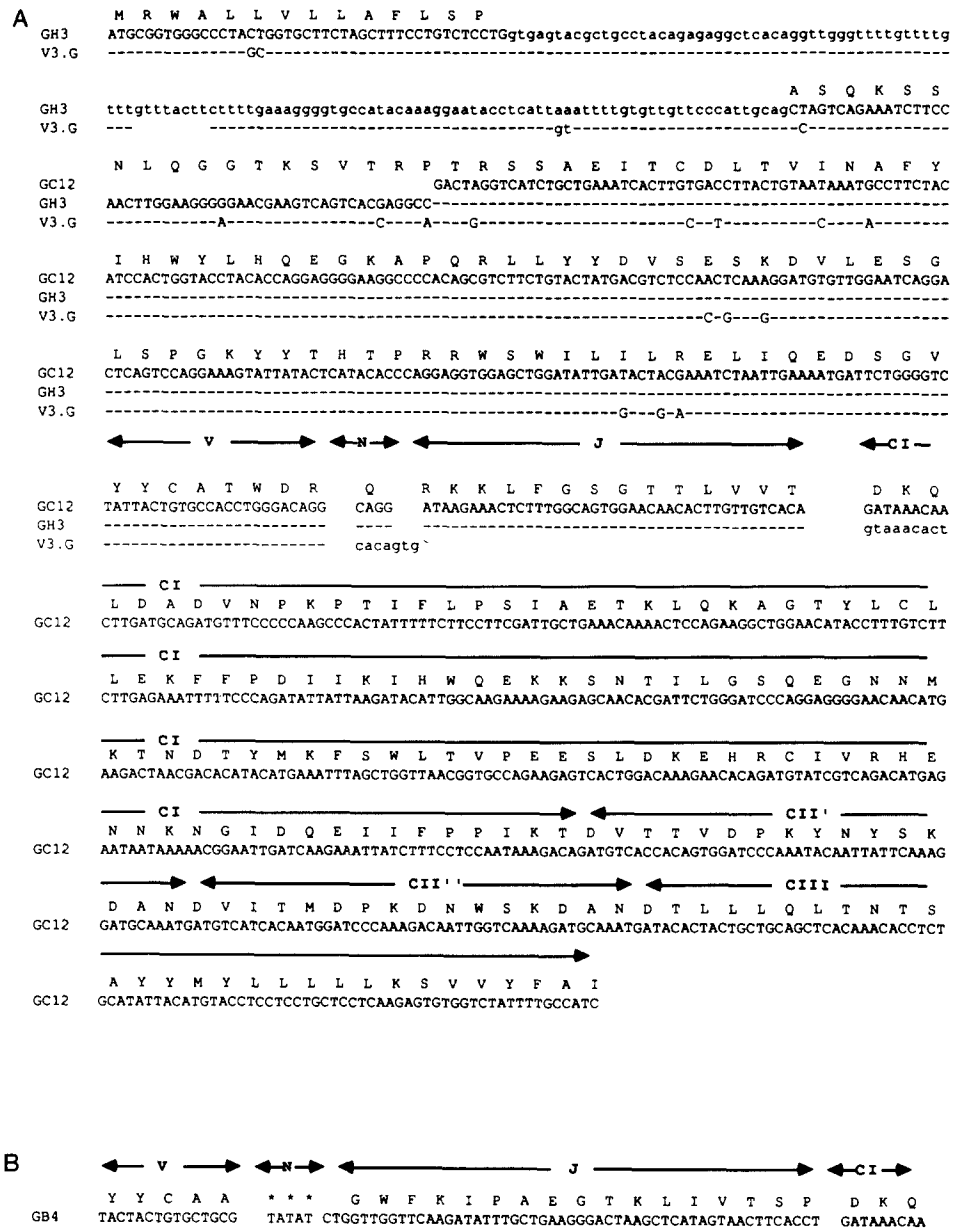

FIGURE 2. Nucleotide sequence of CII γ genes. (*A*) Nucleotide sequence of cDNA (*GC12*) and genomic clones (*GH3*) representing the productive rearranged γ allele of CII. The sequences are compared with a member of the $V_\gamma I$ subfamily (*V3.G*) (8). Identical bases between the compared sequences are indicated with dashes. Intron regions are denoted in lower case. (*B*) Nucleotide sequence of a cDNA encoding nonfunctional TCR-γ transcript (*GB4*). This sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00814.

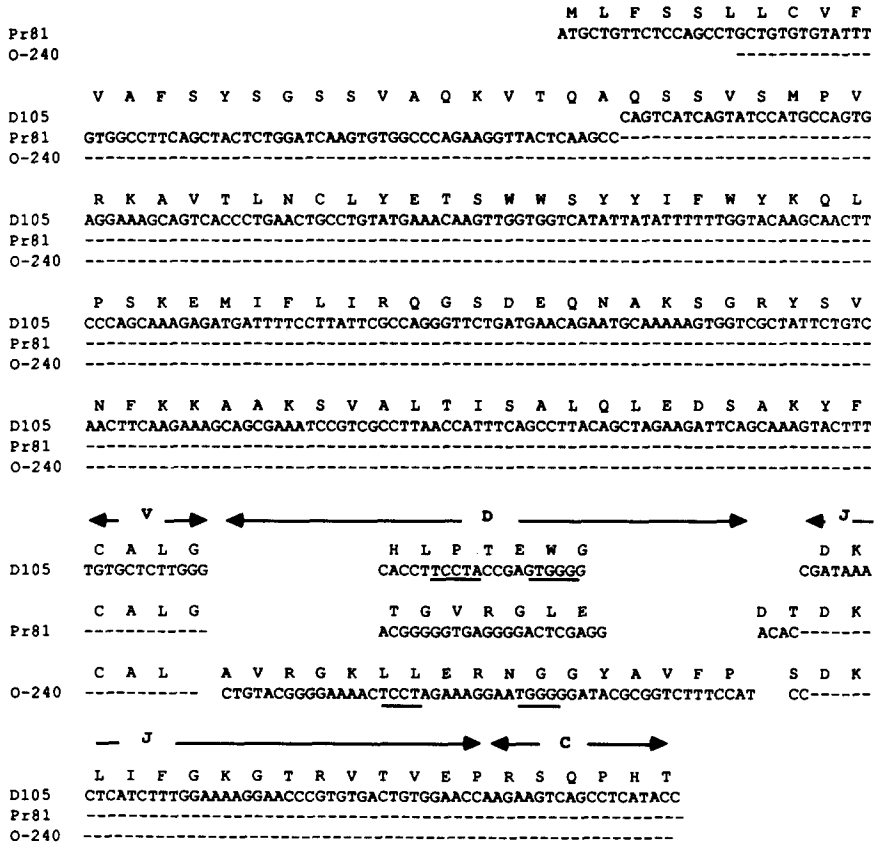

FIGURE 3. Nucleotide sequence of CII TCR-δ cDNA. The nucleotide sequence of CII δ cDNA clone D105 is compared with the sequence of δ cDNA clones isolated from PEER (*Pr81;17*) and IDP2 (*0-240;11*). Dashes indicate identical nucleotides between compared sequences. Undefined nucleotides between putative V and J segments may represent D segments, N regions, or both; shared sequences in these regions that may reflect D segments are underlined. This sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00814.

cell line (IDP2) and a T cell leukemia (Peer) (8, 12); although the junctional regions are strikingly different (Fig. 3). In addition, a large fraction of TCR-γ/δ-bearing fetal thymocytes also were found to express this Vδ segment (12). The significance of the apparently frequent utilization of this V segment in a large number of normal and malignant T cells remains to be determined. In the murine system, it has been suggested that there is a limited repertoire of V elements and that diversity among δ chains is primarily junctional in nature (13); the current human findings are consistent with that possibility. However, additional studies of V gene utilization in different human γ/δ T cell subsets will be necessary to confirm the limited germline repertoire.

Summary

The $CD3^+$, IL-2-dependent normal human thymocyte clone, CII, expresses on its surface a CD3-associated γ/δ TCR. We have further elucidated the structure of

this receptor from the nucleotide sequence of cDNA and genomic clones from CII that encode functional TCR-γ and -δ chains. We find that the CII line expresses a $C_\gamma 2$ constant region that is a polymorphic form lacking a copy of an internal exon; the sequence of this constant region accounts for the size of the γ chain and non-covalent linkage of γ and δ chains in the CII TCR. The V_δ region used for the CII TCR is identical to the several previously characterized expressed human V_δ segments. Possible implications of this finding are discussed.

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