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, noncovalently 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 Jγ probe pH60 was kindly provided by T. H. Rabbits (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.
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 cytofluorography. 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 Jγ1 probe (pH60). The 5' region of the Cγ1 and Cγ2 genes, respectively, are contained on germline pH60-hybridizing Bam HI fragments of ~18 and ~14 kb (Fig. 1 A, lane B4). 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 Cγ1 on both alleles, and maintained the germline configuration for Cγ2 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 Jδ1/Cδ-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 (B4 and LD), human T cell leukemia line (RPMI8402), or CII was assayed by genomic blotting procedures for hybridization to the Jγ1- and Jγ2-hybridizing probe pH60. The map of the genomic Jγ region, as previously reported (8), is indicated below (B) 10 μg genomic DNA was digested with Eco RI and assayed for hybridization to 32P-labeled Jγ-Cγ-containing cDNA probe. The map of the Jγ-Cγ region is indicated below as previously reported (10). Regions hybridizing to the probe are indicated in black.
B cell DNA contained three Eco RI fragments of \( \sim 6, \sim 3.5, \) and \( \sim 1.9 \) kb that hybridized to the \( \delta \)-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 \( J_\delta \), as the probe has 5' but not 3' flanking germline sequences of \( J_\delta \) (Fig. 1 B, bottom; 7). Thus, both the \( C_\gamma \) and \( C_\delta \) loci are rearranged in CII.

**Isolation of the TCR-\( \gamma \) cDNA and Genomic Clones.** To characterize the TCR-\( \gamma \) RNA transcripts from CII cells, we isolated \( C_\gamma \)-hybridizing cDNA clones from a CII cDNA library. Two distinct \( V_{\gamma 2}C_\gamma \)-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 \( \gamma \) allele (Fig. 2 A, GCI2). To obtain the complete \( V \gamma \) 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 \( V_{\gamma 1} \) family (Fig. 2 A, reference 9). Another cDNA sequence contained a \( V_{\gamma 3} \) \( III \) region joined via \( J_\gamma P 1 \) (9) to \( C_\gamma 1 \) but in a different translational reading frame, indicating that it represented the nonproductively rearranged \( \gamma \) 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-\( \gamma \) 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-\( \gamma/\delta \) chains (used in the nonproductive CII rearrangement; 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 \( \gamma \) rearrangement is consistent with the observation that the \( \gamma \) and \( \delta \) 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-\( \gamma \) peptide expressed by CII is smaller than the \( \gamma \) 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 CII polymorphism was found to occur more frequently in the population (10), CII is the first normal T cell line reported to express a \( \gamma \) 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 \( \delta \) Chain.** To further characterize the CII TCR \( \delta \) chain, we isolated \( C_\delta \)-hybridizing cDNA clones from a CII cDNA library. The nucleotide sequence of one such clone (DI05) reveals a \( V_\delta \) gene segment productively joined to \( J_\delta \) (Fig. 3); this \( V_\delta \) segment is identical to a previously characterized single copy \( V \) gene (8, 12). The \( V \) and \( J \) segments of DI05 are joined by a 20-bp region that cannot be attributed to known TCR-\( \delta \) V or J segments (7, Fig. 3). These extra nucleotides could potentially accommodate a D segment, perhaps more than one, as in murine TCR-\( \delta \) rearrangements (13) (Fig. 3). The TCR-\( \delta \) 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 Vγ1 subfamily (V3.G) (B). 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.
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 C2 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 Vδ region used for the CII TCR is identical to the several previously characterized expressed human Vδ segments. Possible implications of this finding are discussed.

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