recent purification and sequence data of ANF receptors indicate that there are two biochemically distinct classes of ANF binding sites [J. Lewicki *et al.*, in *Proceedings and Abstracts of the Second World Congress of Biologically Active Atrial Peptides*, American Society of Hypertension, New York, 16–21 May 1987 (American Society of Hypertension, New York, 1987), p. 184]. Thus, C-ANF receptors are not simply "spare" receptors of a single class of ANF receptors.
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Identification of Putative Human T Cell Receptor δ Complementary DNA Clones

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A novel T cell receptor (TCR) subunit termed TCR δ, associated with TCR γ and CD3 polypeptides, was recently found on a subpopulation of human T lymphocytes. T cell–specific complementary DNA clones present in a human TCR γδ T cell complementary DNA library were obtained and characterized in order to identify candidate clones encoding TCR δ. One cross-hybridizing group of clones detected transcripts that are expressed in lymphocytes bearing TCR γδ but not in other T lymphocytes and are encoded by genes that are rearranged in TCR γδ lymphocytes but deleted in other T lymphocytes. Their sequences indicate homology to the variable, joining, and constant elements of other TCR and immunoglobulin genes. These characteristics, as well as the immunochemical data presented in a companion paper, are strong evidence that the complementary DNA clones encode TCR δ.

THE ANTIGEN-SPECIFIC RECEPTOR on the surface of most peripheral blood T lymphocytes is a disulfide-linked heterodimer composed of α and β subunits (40 to 50 kD), noncovalently associated with CD3 polypeptides (*1–3*). The TCR α and TCR β polypeptides are encoded by immunoglobulin-like variable (V), diversity (D), joining (J), and constant (C) gene segments that rearrange to form a functional gene during thymic T cell maturation (*4–8*). The TCR γ gene was identified as an additional immunoglobulin-like, T cell rearranging gene (*9*). Recently, 40- to 55-kD polypeptides encoded by functionally rearranged TCR γ genes were identified as one component of a heterodimer, associated with CD3 polypeptides, on populations of peripheral blood T lymphocytes (*10*), thymic T cells (*11*), and dendritic epidermal cells (*12*). The second component of the heterodimer appeared distinct from TCR γ and was proposed as a novel TCR subunit termed TCR δ.

Although little information is available concerning the biochemistry of the TCR δ protein, it might be supposed that it bears structural homology with other TCR subunits. This supposition leads to the prediction that TCR δ may be encoded by a gene that displays significant sequence homology to members of the immunoglobulin gene superfamily, that rearranges during T cell maturation, and that is expressed specifically in TCR γδ T cells. Guided by these assumptions, we sought to obtain and characterize T cell–specific complementary DNA (cDNA) clones generated from messenger RNA (mRNA) of the human TCR γδ cell line IDP2 (*10*).

A T cell–specific cDNA probe was generated by synthesizing ^{32}P-labeled first-strand cDNA of high specific activity from IDP2 polyadenylated [poly(A)$^+$] RNA, and subjecting this material to two cycles of hybridization with poly(A)$^+$ RNA from the human B cell line JY followed by hydroxylapatite chromatography (*13*). The twice subtracted single-stranded material was used to probe 40,000 plaques of an IDP2 λgt10 cDNA library (*14*), and 391 (1%) hybridizing plaques were obtained. Subsequent analysis organized these clones into 14 cross-hybridizing groups, composed of as many as 139 and as few as 2 members. Three groups were identified as encoding TCR γ (10 members), TCR β (20 members), and CD3 δ/ε (7 members), as judged by hybridization with appropriate probes. Representative members of the remaining 11 groups (A,B,C,D,E,G,I,K,M,O,R) were labeled with ^{32}P and used to probe Northern blots. One group (O, consisting of six members) detected transcripts expressed in IDP2 and the TCR γδ cell line PEER (*15–17*) but not expressed in JY and the TCR αβ cell line HPB-ALL. On the basis of this result, two group O clones (O-240 and O-254) were selected for further study.

Northern blot analysis of a larger panel of RNA samples with O-240 as a probe (Fig. 1A) revealed the expression of cross-hybridizing transcripts in four TCR γδ cell lines [IDP2, PEER, Molt-13 (*18*), and PBL L1 (*16*)]. Four distinct transcripts, of 2.2, 1.7, 1.3, and 0.8 kb (arrows in Fig. 1A), were detected. However, transcripts were undetectable in B cell line JY, myeloid cell line HL60, the TCR αβ-bearing T cell line

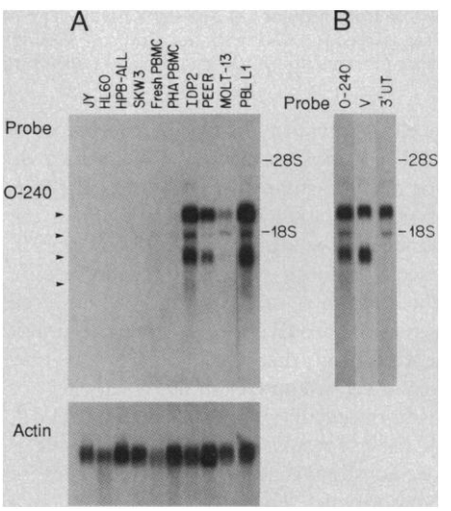

Fig. 1. Northern blot analysis of group O hybridizing transcripts. (**A**) Total RNA samples (5 μg) were electrophoresed through 1.5% agarose gels containing 2.2*M* formaldehyde, transferred to nitrocellulose, and probed with nick-translated O-240 or chicken actin (Oncor). Filters were washed with 1× SSC and 0.5% SDS at 23°C and then with 0.1× SSC at 50°C. RNA sources are: JY, B cell line; HL60, myeloid cell line; HPB-ALL and SKW3, TCR αβ and surface TCR$^-$ T cell lines, respectively; fresh PBMC and PHA PBMC, fresh and 2-day PHA-activated peripheral blood mononuclear cells; IDP2, PEER, Molt-13, and PBL-L1 [identical to WT31$^-$ PBL line (*16*)] TCR γδ T cell lines. (**B**) IDP2 RNA treated as above was probed with nick-translated O-240, a 240-bp Eco RI–Sca I fragment of O-240/38 (V probe; see Fig. 3A) labeled by hexanucleotide priming, or a 550-bp Hae III fragment of O-240 (3′ UT; see Fig. 3A) labeled by nick-translation. Washing was as in (A). Arrowheads mark the positions of the four major transcripts detected; 18*S* and 28*S* ribosomal RNA served as markers.

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HPB-ALL, and T cell line SKW3, which does not express a surface TCR. Transcripts were barely detectable in RNA from fresh or phytohemagglutinin-activated peripheral blood mononuclear cells (PHA-PBMC), of which only a small fraction express TCR γδ.

Analysis of genomic DNA digested with various enzymes revealed no evidence for rearrangement of O-240 hybridizing sequences in TCR γδ T cells. However, although a 9.5-kb Xba I fragment (and a 9.0-kb polymorphic fragment) was detected in B cells, myeloid cells, and TCR γδ T cells, this fragment was deleted on both chromosomes in all other T cells examined (Fig. 2A). This likely represents somatic deletion rather than polymorphism, since pairs of B and T cell lines derived from the same individual were analyzed (SB and HSB; 8392 and 8402). These results suggest that deletion of sequences detected by O-240 may accompany rearrangement at either the TCR α or TCR β locus.

Initial sequence analysis of clones O-240 (1.5 kb) and O-254 (0.7 kb) indicated that they both extended from a natural Eco RI site at the 5′ terminus through a poly(A) tail and an Eco RI linker at the 3′ terminus. These clones derived from a cDNA library constructed without methylation of Eco RI sites. Information 5′ to the natural Eco RI site was obtained by using O-240 to probe an Eco RI methylated IDP2 λgt10 cDNA library. Two clones that spanned the Eco RI site, O-240/38 (1.3 kb) and O-240/47 (1.4 kb), were selected for detailed study. Notably, a probe derived from the 5′ end of O-240/38 detected discrete rearrangements in both Eco RI and Pvu II digests of genomic DNA from five of five TCR γδ cell lines (Fig. 2B). Of the three germline fragments in each digest detected by this probe (arrows in Fig. 2B), rearrangements of the 3.3-kb Eco RI and 23.0-kb Pvu II fragments appeared to be similar in the five TCR γδ cell lines, whereas rearrangements of the 6.6-kb Eco RI and 2.0-kb Pvu II fragments distinguished the different cell lines. In contrast to these discrete rearrangements, a heterogeneous smear of rearrangements was detected in Eco RI digests of two samples of fetal thymus DNA.

The comparative organizations, partial restriction maps, and sequencing strategies used to characterize clones O-240, O-254, O-240/38, and O-240/47 are presented in Fig. 3A. The composite sequence (Fig. 3B) presents a long open reading frame of 293 amino acids and is clearly composed of V-, J- and C-like elements similar to those of TCR and immunoglobulin genes. Strikingly, the putative C-region sequence is 79% identical at the nucleotide level, and 73% identical at the amino acid level, to the sequence of a novel murine TCR constant-region gene (Cx) recently reported by Chien *et al.* (*19*) to reside within the TCR α locus. The high degree of sequence identity indicates that the group O clones reported here define the human homolog of murine Cx. In this light, the deletion of this sequence in TCR αβ T cells is suggestive that the human constant region, like its murine counterpart, maps 5′ to Cα within the human TCR α locus.

The 5′ ends of O-240/38 and O-240/47 define a partial putative leader (L) sequence and a V-region sequence. The precise processing point between these segments that defines the amino terminus of the mature protein is uncertain. However, processing of the TCR α chain in HPB-MLT has been suggested to lie between Ala(−1) and Gln(+1), since the amino terminus of TCR α is blocked (*8*). By analogy, we have tentatively assigned the processing point to this location in our sequence, since in the region from −4 to +8 the two sequences are identical in 11 of 12 residues.

The putative V region displays 57% amino acid sequence identity with a human Vα sequence [PGA5 (*8*)], 26% identity with a human Vβ sequence [YT35 (*4*)], and 21% identity with a human Vγ sequence [Vγ2 (*20*)]. Comparisons among Vα subgroup sequences and among Vβ subgroup sequences can be used to identify consensus residues that occur in 50% or more of Vα or Vβ subgroups. The V-region sequence reported here matches the Vα consensus in 75% of these residues (30 of 40) (Fig. 4A). By contrast, it only matches the Vβ consensus in 49% of these residues (17 of 35). For comparison, the randomly selected Vα sequences 1.1, 6.1, and 12.1 match the Vα consensus in 70, 73, and 73% of these positions, respectively, whereas the Vβ sequences 2.2, 5.4, and 8.1 match at 40, 53, and 60%. Thus this V region is clearly Vα-like, since it is as close to the consensus as other Vα sequences.

Amino acids 112 to 125 display significant homology to human TCR consensus J-region sequences (Fig. 4B) and with the J region associated with murine Cx (Fig. 3B). However, amino acids 94 to 111 are homologous to neither V nor J sequences, and homology with the murine clone is minimal in this region as well (Fig. 3B). Whether and how much of this area might be encoded by a separate D element or might result from so called N-region diversity (*21*) remains to be determined. In any event, the coding sequence remains in frame across the V(D)J

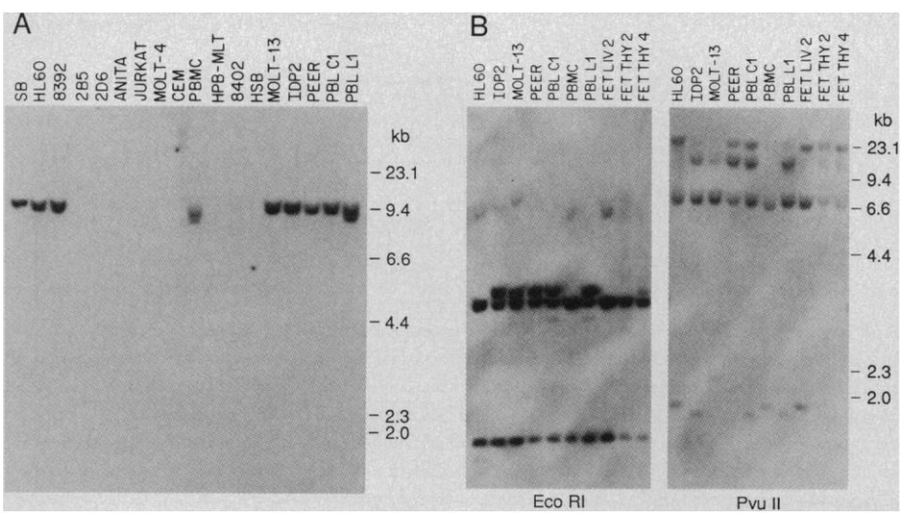

Fig. 2. Southern blot analysis of group O hybridizing genomic DNA. (**A**) High molecular weight genomic DNA samples (5 μg) were digested with Xba I, electrophoresed through 0.7% agarose, transferred to nitrocellulose, and probed with nick-translated O-240. Filters were washed with 1× SSC and 0.5% SDS at 23°C and then with 0.1× SSC and 0.1× SDS at 68°C. DNA sources are: B cell lines SB and 8392; myeloid cell line HL60; TCR αβ T cell lines 2B5 (*35*), 2D6 (*35*), Anita, Jurkat, and HPB-MLT; surface TCR$^-$ T cell lines Molt-4, CEM, 8402, and HSB; fresh peripheral blood mononuclear cells PBMC; and TCR γδ T cell lines Molt-13, IDP2, PEER, and PBL L1. PBMC and PBL L1 are derived from the same individual. The diminished signal in PBMC presumably results from deletion in most T cells in the sample. The remaining signal (largely B cells and monocytes) serves as a germline control for PBL L1. On this basis, the 9.0-kb fragment is interpreted as a polymorphism rather than a rearrangement. A λ Hind III digest was used as markers. (**B**) Genomic DNA samples digested with Eco RI or Pvu II were analyzed as above and probed with the 430-bp 5′ Eco RI fragment of clone O-240/38 (VJC probe; Fig. 3A) labeled by nick-translation. Filters were washed with 1× SSC and 0.5% SDS at 23°C and then with 0.2× SSC and 0.1× SDS at 55°C. PBL C1 is a TCR γδ T cell clone (*16*); FET LIV 2 and FET THY 2 are fetal liver and thymus samples from the same fetus; FET THY 4 is from another fetus; other DNA samples are as in (A). Germline bands in each digest are marked by arrowheads.

Fig. 3. Organization and nucleotide sequence of group O cDNA clones. (**A**) Nucleotide sequences of clones O-240, O-254, O-240/38, and O-240/47 were determined using the dideoxy chain termination method via the outlined strategy. Partial restriction maps and the locations of probes V, VJC, and 3'UT (hatched bars) are presented. Poly(A) tails are noted. (**B**) The composite nucleotide and deduced amino acid sequences of the group O cDNA clones are presented. Amino acid residues are numbered from the presumed amino-terminal processing point, as described in the text. Cysteine residues are boxed, potential *N*-linked glycosylation sites are bracketed, and polyadenylation signals used in O-240 and O-254 are underlined. O-240/38 begins within codon 7 of the composite sequence, whereas O-240 and O-254 begin within codon 150. Within the coding region, sequences agree at all positions except for codon 161 (GTG in O-254 and O-240/38, TTG in O-240). This discrepancy is presumed to result from a reverse transcriptase error in O-240. The composite nucleotide sequence is compared with that of the coding region of murine cDNA clone DN-4 (*19*). A dash denotes identity and an asterisk denotes a gap.

junction, and hence the IDP2 group O cDNA clones correspond to transcripts from a productively rearranged gene.

The putative constant-region sequence includes an immunoglobulin-like region with two cysteine residues separated by 51 amino acids, a connector region carrying a cysteine residue that is typically thought to mediate interchain disulfide bonds between TCR chains, and an intramembraneous region. Two potential sites of *N*-linked glycosylation are situated immediately amino terminal to the first cysteine and carboxyl terminal to the second cysteine. Within the first 91 amino acids of the constant region, amino acid sequence identity is highest with Cγ (*22*) and Cλ (*23*) (22 and 20%, respectively) and lower with Cα (*24*) and Cβ (*25*) (15 and 11%, respectively). The connector region shares elements with each of the other TCR chains. However, the 40 amino acids including and flanking the presumed transmembrane region show a significantly higher number of identities with the homologous region of Cα (30%) than with either Cβ (8%) or Cγ (13%) (Fig. 5A). These relationships are dramatically underscored by comparison of the number and distribution of charged and uncharged residues throughout this region (Fig. 5B). Like Cα, this constant region appears to have at least two positively charged residues that may be buried within the membrane. Such charged residues are thought to be important in mediating interactions with CD3 components, which display acidic residues within their transmembrane regions (*26–28*). Also as in Cα, an intracellular tail, if it exists at all, is extremely short. Whereas Cβ and Cγ display putative intracellular tails that are highly charged, the IDP2 group O sequence displays a single basic residue followed by four hydrophobic amino acids. The corresponding Cα sequence is of equal length. Regardless of how the membrane proximal sequences are disposed relative to the lipid bilayer and to CD3 components, it appears likely that this portion of the constant region is involved in interactions highly analogous to those of Cα.

The 3' untranslated (3'UT) sequences indicate the use of alternative polyadenylation sites. Whereas the O-240 3' UT extends some 1050 bp through an ATTAAA polyadenylation signal, that of O-254 extends only 260 bp, with polyadenylation following the sequence TATAAA. Both sequences differ from the consensus AATAAA by a single nucleotide. Potential for additional heterogeneity exists, since the sequence TATAAA occurs twice more within the O-240 sequence (19 bp on the 3' side of the signal used in O-254 and 133 bp on the

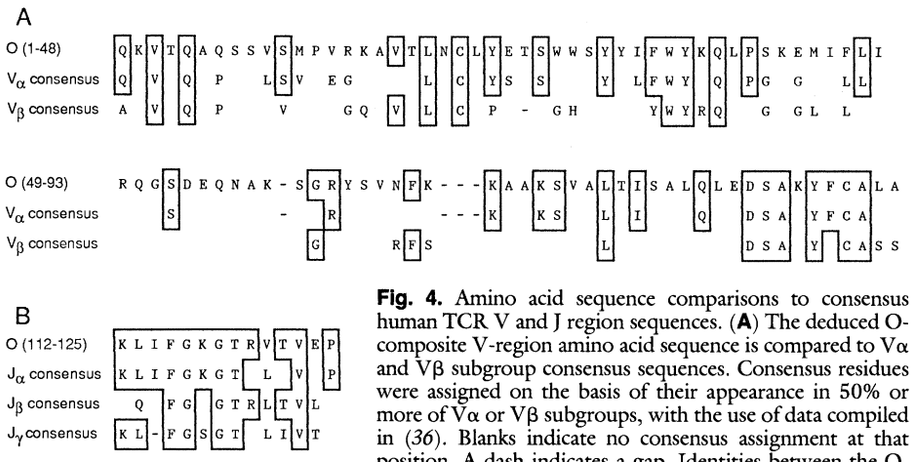

Fig. 4. Amino acid sequence comparisons to consensus human TCR V and J region sequences. (**A**) The deduced O-composite V-region amino acid sequence is compared to Vα and Vβ subgroup consensus sequences. Consensus residues were assigned on the basis of their appearance in 50% or more of Vα or Vβ subgroups, with the use of data compiled in (*36*). Blanks indicate no consensus assignment at that position. A dash indicates a gap. Identities between the O-composite sequence and consensus residues are boxed. (**B**) The deduced O-composite J-region amino acid sequence is compared to Jα, Jβ, and Jγ consensus residues. Consensus residues were assigned on the basis of their appearance in 40% or more of the Jα, Jβ, and Jγ sequences compiled in (*36*) and (*37*).

5′ side of the signal used in O-240). Variation in the site of polyadenylation is at least partially responsible for the transcript heterogeneity observed on Northern blots (Fig. 1B). Whereas the 2.2- and 1.3-kb transcripts are selectively detected by a V-region probe, an O-240–specific 3′ UT probe detects only the 2.2- and 1.7-kb transcripts. Thus in IDP2, PEER, and PBL L1 the two most abundant species (2.2 and 1.3 kb) represent differentially polyadenylated transcripts. The minor 1.7- and 0.8-kb species therefore represent transcripts lacking V regions and are presumably transcribed from partially rearranged genes. By contrast, TCR β mRNA heterogeneity primarily results from the latter mechanism (*29*).

By all accounts, the group O cDNA clones appear to be good candidates to encode the IDP2 TCR δ peptide. They detect transcripts that are expressed specifically in TCR γδ T cells and are encoded by genes specifically rearranged in the same cells. Transcript levels correlate well with the level of expression of cell surface TCR δ polypeptide, which is lower in PEER than in IDP2, and lower still in Molt-13 (*18*). Furthermore, they are composed of V, J, and C elements that are homologous to those of other TCR and immunoglobulin genes. The cDNA clones derived from IDP2 mRNA remain in frame across the V-J junction, indicating that they would encode a functional polypeptide in these cells. The cDNA sequence predicts a polypeptide backbone size of 31.3 kD, with two potential sites for *N*-linked glycosylation. As demonstrated in the accompanying paper (*30*), these predictions agree well with the properties of the TCR δ peptide on IDP2 cells. Furthermore, it is demonstrated in that study by in vitro transcription and translation analysis that clone O-240/38 encodes a polypeptide that is specifically recognized by a monoclonal antibody directed against TCR δ.

Human TCR γ and δ peptides can be found either in a disulfide-linked form or an unlinked form on different cell lines (*16, 31–33*). This structural heterogeneity is controlled, at least in part, by TCR γ C-region usage, since the Cγ-1 gene encodes a cysteine in the membrane proximal connector region that is absent in Cγ-2 (*14, 17, 22*). IDP2 uses the Cγ-2 gene, lacks this cysteine, and displays a non-disulfide-linked receptor on the cell surface (*14, 16*). One might have predicted that the IDP2 TCR δ peptide would lack the analogous cysteine as well. However, our cDNA sequences predict that IDP2 TCR δ carries a cysteine in the membrane proximal connector that would be available for disulfide linkage. Moreover, Southern blots (Fig. 2A) provide evidence for only a single TCR δ C-region gene. Thus, it appears that a single TCR δ gene product could pair with TCR γ peptides encoded by Cγ-1 to form a disulfide-linked complex, or with TCR γ peptides encoded by Cγ-2 to form a nonlinked complex.

In contrast to the large number of V gene segments available for TCR α and TCR β, there are only a limited number of functional TCR γ V gene segments (*20*). Thus the size of the TCR δ V gene pool will be important in determining the number of antigens that may be recognized by TCR γδ lymphocytes. The V region used by IDP2 is clearly related to TCR α V regions, but whether TCR α and TCR δ draw from the same or distinct pools of V genes is not known. Strikingly, nucleotide sequence analysis indicates that the IDP2, PBL C1, and Molt-13 TCR δ chains all use the same V gene (*34*), a conclusion consistent with genomic rearrangement data (Fig. 2B). This result might suggest a limited TCR δ V repertoire. The size and nature of the TCR δ V gene pool will have intriguing implications for the relationship betwen the TCR αβ and TCR γδ repertoires and will be an important area for further study.

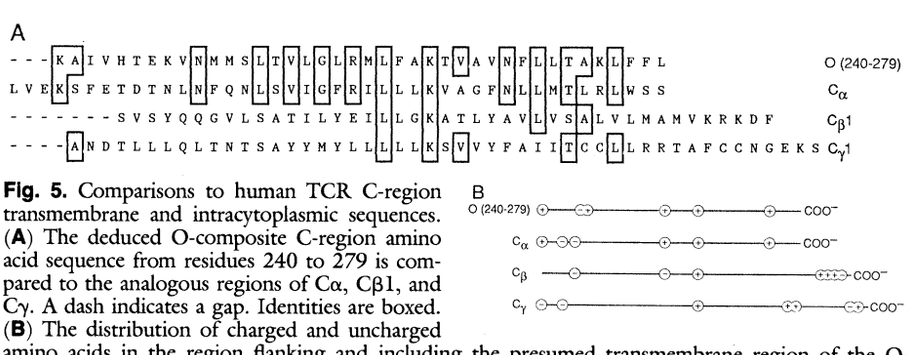

Fig. 5. Comparisons to human TCR C-region transmembrane and intracytoplasmic sequences. (**A**) The deduced O-composite C-region amino acid sequence from residues 240 to 279 is compared to the analogous regions of Cα, Cβ1, and Cγ. A dash indicates a gap. Identities are boxed. (**B**) The distribution of charged and uncharged amino acids in the region flanking and including the presumed transmembrane region of the O-composite sequence is compared with those of Cα, Cβ2, and Cγ1.

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Immunochemical Proof That a Novel Rearranging Gene Encodes the T Cell Receptor δ Subunit

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The T cell receptor (TCR) δ protein is expressed as part of a heterodimer with TCR γ, in association with the CD3 polypeptides on a subset of functional peripheral blood T lymphocytes, thymocytes, and certain leukemic T cell lines. A monoclonal antibody directed against TCR δ was produced that binds specifically to the surface of several TCR γδ cell lines and immunoprecipitates the TCR γδ as a heterodimer from Triton X-100 detergent lysates and also immunoprecipitates the TCR δ subunit alone after chain separation. A candidate human TCR δ complementary DNA clone (IDP2 O-240/38), reported in a companion paper, was isolated by the subtractive library approach from a TCR γδ cell line. This complementary DNA clone was used to direct the synthesis of a polypeptide that is specifically recognized by the monoclonal antibody to TCR δ. This complementary DNA clone thus corresponds to the gene that encodes the TCR δ subunit.

T CELL RECEPTOR (TCR) α and β genes rearrange in T cells and encode the variable part of the TCR αβ–CD3 complex. Unexpectedly, another gene, TCR γ (*1*), that rearranges specifically in T cells was also found. The search for the protein product of this gene led to the identification of a second T cell receptor composed of the TCR γ polypeptide, the CD3 proteins, and a previously unidentified polypeptide called TCR δ (*2*). This receptor is expressed on the cell surface of a subpopulation of functional peripheral blood lymphocytes (*3, 4*), thymocytes (*5–7*), and other cell types (*8–10*). To understand better the structure, diversity, and function of TCR γδ, we sought to characterize the TCR δ subunit. The TCR δ protein is serologically distinct from the TCR γ protein since it is not recognized by antisera raised against TCR γ–encoded sequences (*2, 6*). Moreover, the tryptic peptide map of TCR δ is distinct from that of TCR γ protein isolated from the same cell line (*11*).

We generated a monoclonal antibody (mAb) that is specifically reactive with the TCR δ subunit and we determined the size and extent of glycosylation of the TCR δ polypeptide. We present immunochemical evidence that a novel complementary DNA (cDNA) clone encodes the δ subunit of the T cell receptor.

The TCR γδ–CD3 complex from the PEER cell line (*4, 12*) was used as immunogen in the production of antibody-secreting hybridoma cell lines (Fig. 1). Hybridomas were screened both by surface binding in cytofluorographic analysis and by immunoprecipitation in SDS–polyacrylamide gel electrophoresis (SDS-PAGE) analysis of PEER cells. Two hybridoma supernatants (5A6 and 4A1) bound to the surface of PEER cells. After subcloning, one mAb (5A6.E9) was characterized further. This mAb bound to the surface of TCR γδ lymphocytes (PEER and IDP2) but failed to react with TCR αβ cells (HPB-MLT or JURKAT) or with non-T leukocytes (Fig. 1). Although the immunogen was composed of a complex of TCR γδ and CD3, the differential reactivity on TCR γδ cell lines suggested the mAb was not directed against CD3 determinants.

The specificity of the mAb was determined in immunoprecipitation studies under conditions that affect the association of the chains constituting the complex. For example, when ^{125}I-labeled IDP2 cells are solubilized in CHAPS detergent, TCR γ and δ and CD3 γ, δ, and ε subunits remain part of an associated complex immunoprecipitated by an antibody directed against CD3 (Fig. 2, lanes 3, and 4). However, if radiolabeled IDP2 cells are solubilized in 2% TX-100 detergent, TCR γδ and CD3 are largely dissociated, and immunoprecipitation with a mAb to CD3 results in selective isolation of the CD3 chains (Fig. 2, lane 5). Correspondingly, under these conditions mAb 5A6.E9 immunoprecipitates TCR γδ as a heterodimer, without associated CD3 chains (Fig. 2, lane 6). This observation provides the first direct evidence that TCR γ and TCR δ exist as a heterodimer even in

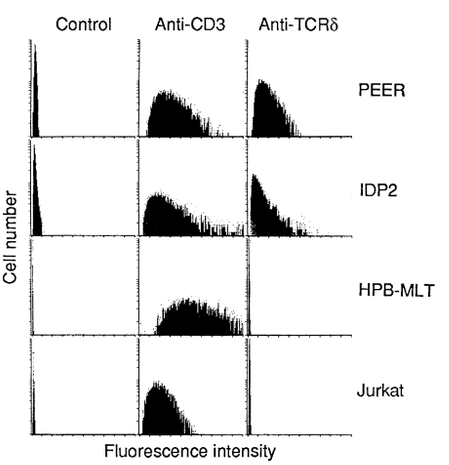

Fig. 1. Cytofluorographic analysis of T cell lines with anti-TCR δ1. TCR γδ cell lines PEER and IDP2 or TCR αβ cell lines HPB-MLT and JURKAT were stained with anti-TCR δ1 (Ig G1) culture supernatant, then with fluorescein isothiocyanate–conjugated goat antiserum to mouse Ig F(ab)′$_2$ fragments, and then analyzed on an Ortho cytofluorograph as described earlier (*2*). Control was the mAb secreted by P3X63.Ag8 hybridoma (P3), and the mAb to CD3 was anti-Leu 4 (*18*). The mAb to TCR δ was made as follows. PEER cells (1 g) were solubilized in 50 ml of 0.3% CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate) detergent and were immunoprecipitated with 1 μl of UCHT1 (*19*) ascites, 500 μl of mAb 187.1 culture supernatant, and 125 μl of a 10% suspension of fixed *Staphylococcus aureus* Cowan I strain for each immunization. Four intraperitoneal injections at 6-week intervals were carried out and were followed by a final boost of TCR γδ (without CD3) isolated by selective elution from the immune complexes with 2.5% TX-100 in 0.65*M* NaCl. After dilution to isotonic salt concentration, the eluted material was administered both intravenously and intraperitoneally, and 4 days after this final injection, the mice were killed and fusion was carried out as described earlier (*20*).

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