

Description

1. INTRODUCTION

The present invention is directed to a form of the human γ T cell antigen receptor polypeptide termed Form 2bc, which has a molecular weight of about 40,000 daltons, and a constant region which contains a sequence encoded by only two C γ 2 CII exon copies. The invention also relates to T cell antigen receptor heterodimers comprising γ Form 2bc, and to nucleic acid sequences encoding γ Form 2bc and portions thereof. The invention also provides monoclonal antibodies specifically reactive with an epitope of the γ or δ T cell antigen receptor polypeptides.

2. BACKGROUND OF THE INVENTION

The T cell antigen receptor (TCR) was shown to be a clone specific disulfide-linked heterodimer on T cells, composed of two glycosylated subunits, one of which is designated the α chain and the other of which is designated the β chain. The α and β TCR subunits have a relative molecular mass (M_r) of approximately 50,000 and 40,000 daltons, respectively (Allison et al., 1982, Immunol. 129:2293-2300; Meuer et al., 1983, J. Exp. Med. 157:705-719; Haskins et al., 1983, J. Exp. Med. 157:1149-1169). Genes that rearrange during T cell ontogeny and encode the β TCR (Yanagi et al., 1984, Nature 308:145-149; Hedrick et al., 1984, Nature 308:153-158) and α TCR (Chien et al., 1984, Nature 312:31-35; Saito et al., 1984, Nature 312:36-40; Sim et al., 1984, Nature 312:771-775) subunits were isolated either by subtractive hybridization or by probing with oligonucleotides.

The alpha and beta chains of the T cell antigen receptor of a T cell clone are each composed of a unique combination of domains designated variable (V), diversity (D), joining (J), and constant (C) (Siu et al., 1984, Cell 37:393; Yanagi et al., 1985, Proc. Natl. Acad. Sci. USA 82: 3430). Hypervariable regions have been identified (Patten et al., 1984, Nature 312:40; Becker et al., 1985, Nature 317:430). In each T cell clone, the combination of V, D and J domains of both the alpha and the beta chains participates in antigen recognition in a manner which is uniquely characteristic of that T cell clone and defines a unique binding site, also known as the idiotype of the T cell clone. In contrast, the C domain does not participate in antigen binding.

A unique feature of the human α , β TCR was the observed comodulation (Meuer et al., 1983, J. Exp. Med. 157:705-719), colimmunoprecipitation (PCT International Publication No. WO 88/00209, published January 14, 1988; Oettgen, et al., 1984, J. Biol. Chem. 259:12,039-12,048) and required coexpression (Weiss et al., 1984, J. Exp. Med. 160:1284-1299) of the α , β TCR molecules with a CD3 glycoprotein complex. Subsequently, the direct physical association of the two protein complexes was demonstrated by chemically cross-linking the α , β TCR molecules to the T3 glycoprotein and identifying the components of the cross-linked complex as the TCR subunit and the T3 glycoprotein (M_r 28,000) subunit (Brenner et al., 1985, Cell 40:183-190). A T3 counterpart is similarly associated with murine α , β TCR (Allison et al., 1985, Nature 314:107-109; Samelson et al., 1984, Immunol. Rev. 81:131-144).

A third gene that rearranges in T cells, designated γ TCR, was identified, first in mice (Saito et al., 1984, Nature 309:757-762; Kranz et al., 1985, Nature 313:762-755; Hayday et al., 1985, Cell 40:259-269) and then in humans (Lefranc et al., 1985, Nature 316:464-466; Murre et al., 1985, Nature 316:549-552). The human γ TCR locus appears to consist of between five and ten variable, five joining, and two constant region genes (Dialynas et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83: 2619). Although the total number of functional variable and joining regions is limited, significant diversity is introduced during the process of V-J joining (Kranz et al., 1985, Nature 313:752-755; Lefranc et al., 1986, Cell 45:237-246; Quertermaus et al., 1986, Nature 322:184). The γ TCR gene rearrangements occur in lymphocytes with suppressor-cytotoxic as well as helper phenotypes (Lefranc et al., 1985, Nature 316:464-466; Murre et al., 1985, Nature 316:549-552, Quertermaus et al., 1986, Science 231:252-255; Lefranc et al., 1986, Cell 45:237-246, Iwamoto et al., 1986, J. Exp. Med. 163:1203-1212; Zauderer et al., 1986, J. Exp. Med. 163:1314-1318).

The products of the γ TCR gene have been identified in T3 colimmunoprecipitates from α β TCR⁺CD3⁺ T (Brenner et al., 1986, Nature 322:145-149; Bank et al., 1986, Nature 322:179-181; Borst et al., 1987, Nature 325, 683-688; Moingeon et al., 1987, Nature 325, 723-726, PCT International Publication No. WO 88/00209, published January 14, 1988). The γ TCR polypeptides were identified by use of monoclonal antibodies directed against γ TCR peptide sequences; these polypeptides were found to be incorporated into heterodimers with another polypeptide called δ TCR (Brenner et al., 1986, Nature 322:145-149). The γ , δ heterodimer was reported to be associated noncovalently with CD3.

Use of antisera directed against γ TCR-specific peptides has led to the identification of CD3-associated γ TCR polypeptides on cells originating in peripheral blood, thymus, and a leukemic cell line (Brenner et al., 1986, Nature 322:145-149; Bank et al., 1986, Nature 322:179-181, Weiss et al., 1986, Proc. Natl. Acad. Sci.

U.S.A. 83:6998-7002; Brenner et al., 1987, Nature 325:689-694; Lew et al., 1986, Science 234:1401-1405). Bank et al. (*supra*) disclosed a 44 kd γ form which was associated with a 62,000 kD peptide and T3 on the surface of a human thymocyte clone. A similar γ TCR polypeptide was also identified on murine T lymphocytes, and the expression of this peptide during thymocyte differentiation is the subject of much current study (Roulet et al., 1985, Nature 314:103-107; Snodgrass et al., 1985, Nature 315:232-233; Lew et al., 1986, Science 234:1401-1408; Pardau et al., 1987, Nature 326:79-81; Bluestone et al., 1987, Nature 326:82-84).

With the study of γ TCR⁺ human cell lines, two different γ TCR polypeptides have been identified that differ in their molecular weight and in their ability to form disulfide linkages (Borst et al., 1987, Nature 325:683-688; Brenner et al., 1987, Nature 325:689-694; Moingeon et al., 1987, Nature 325:723-726; Lanier et al., 1987, J. Exp. Med. 165:1076). Two different γ TCR constant region gene segments, called C γ 1 and C γ 2, respectively, have been compared; a cysteine residue encoded by the second exon of C γ 1 appears to be absent in C γ 2 exon segments, and its absence has been suggested to explain the inability of some γ TCR peptides to form disulfide bonds (Kragel, et al., 1987, Science, 237:1051-1055; Littman et al., Nature 326:85088).

In contrast to the multiple forms of γ TCR, the δ TCR molecule is relatively invariant and it appears that there is only one δ TCR constant region (Hata et al., 1987, Science 238:678-682).

During T cell ontogeny, it has been shown that γ TCR gene rearrangement precedes β and α TCR gene rearrangement (Roulet et al., 1985, Nature 314:103-107; Snodgrass et al., 1985, Nature 315:232-233; Sangoter et al., 1986, J. Exp. Med. 163:1491-1508).

Of mature, circulating T lymphocytes, a relatively small proportion are $\gamma\delta$ TCR⁺, and exhibit either CD3⁺4⁻8⁻ (double negative) or CD3⁺4⁻8⁺ surface antigens. CD3⁺4⁻8⁻ T cells constitute approximately two percent of mature CD3⁺ T cells. Unlike most mature CD3⁺4⁺ or CD3⁺8⁺ major histocompatibility locus (MHC) restricted cytotoxic T cells, but similar to CD3⁻ natural killer cells, $\gamma\delta$ TCR⁺ CD3⁺4⁻8⁻ cloned lymphocytes have been shown to exhibit MHC-nonrestricted cytolytic activity; however, unlike natural killer cells, these $\gamma\delta$ ⁺ CD3⁺ 4⁻ 8⁻ T cells did not consistently kill natural killer cell targets, such as K-562 (Borst et al., 1987, 325:683-688; Brenner et al., 1987, Nature 325:689-694; Moingeon et al., 1987, Nature 325:723-726; Bluestone et al., 1987, Nature 326:82-84).

3. SUMMARY OF THE INVENTION

The present invention is directed to a form of the human γ T cell antigen receptor (TCR) polypeptide termed Form 2bc. The Form 2bc γ TCR chain has a primary amino acid sequence substantially as depicted in Figure 14. The Form 2bc γ TCR chain has a molecular weight of about 40,000 daltons, and comprises a constant region containing a sequence encoded by only two C γ 2 CII exon copies. The invention also relates to TCR heterodimers comprising the γ TCR polypeptide Form 2bc.

The present invention provides a purified polypeptide comprising an amino acid sequence of a constant region of a gamma T cell antigen receptor Form 2bc encoded by nucleotide numbers 439 through 1008 of Figure 14. The purified polypeptide has, in particular, a molecular weight of about 40,000 daltons and a sequence comprising a constant region consisting essentially of the amino acid sequence encoded by nucleotide numbers 439 through 1008 of Figure 14, and a variable region.

The present invention also provides a purified polypeptide comprising a gamma T cell antigen receptor Form 2bc having a primary amino acid sequence substantially as depicted in Figure 14 for the variable, N, joining, and constant regions.

The present invention provides nucleic acid sequences encoding the polypeptides of the present invention.

The present invention also provides a monoclonal antibody reactive with an epitope of a variable or rearranged variable-joining region of a δ chain of the human T cell antigen receptor, a monoclonal antibody reactive with an epitope of the constant region of the δ chain of the human T cell antigen receptor, and a monoclonal antibody reactive with an epitope of the constant region of the Form 2bc gamma chain of the human T cell antigen receptor, which epitope is characteristic of the Form 2bc gamma chain.

The invention also relates to monoclonal antibodies specifically reactive with an epitope of the gamma or δ TCR polypeptides. Such antibodies can be identified by detecting their ability to co-modulate the CD3 antigen on a cell which expresses both the gamma,delta TCR and a CD3 antigen. In a specific embodiment, the invention provides a monoclonal antibody reactive with an epitope of a variable or rearranged variable-joining region of the δ TCR chain. In a particular embodiment, such an antibody can be used to detect functional δ TCR variable gene rearrangements in a cell. In another embodiment, the invention provides a monoclonal antibody reactive with an epitope of the constant region of the δ TCR polypeptide. In yet another embodiment, the invention provides a monoclonal antibody reactive with an epitope of the constant region of the Form 2bc gamma chain of the human T cell antigen receptor, which epitope is characteristic of the Form 2bc gamma chain.

The present invention also provides a purified polypeptide complex comprising a T cell antigen receptor

heterodimer consisting of the gamma T cell antigen receptor polypeptide of claim 17 and a second T cell antigen receptor polypeptide selected from the group consisting of the α , β , gamma, and δ T cell antigen receptor polypeptides.

5 The present invention further provides a method for producing a gamma,delta T cell antigen receptor heterodimer which comprises culturing a cell (a) capable of expressing a nucleic acid encoding a δ T cell antigen receptor polypeptide, and (b) which has been transfected with a nucleic acid encoding a gamma T cell antigen receptor polypeptide, under conditions such that both the gamma T cell antigen receptor polypeptide and the δ T cell antigen receptor polypeptide are expressed by the cell and assemble to form a heterodimer.

10 The present invention also provides a method for producing at least a portion of a gamma,delta T cell antigen receptor heterodimer, which comprises culturing a transfected cell (a) capable of expressing a nucleic acid encoding at least a region of a gamma T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, and a joining region; and (b) capable of expressing a nucleic acid encoding at least a region of a δ T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, a joining region, and a diversity region; and subjecting the cell to conditions such that both nucleic acid sequences are expressed by the cell.

The nucleic acid encoding at least a region of the gamma TCR may be transformed into the cell.

The present invention also provides cells that express at least a portion of the gamma,delta TCR.

The present invention, in addition, provides a method for determining the relative usage of a gamma T cell antigen receptor polypeptide in a sample from a human subject comprising:

- 20 (a) determining in a sample containing T cells from a subject the amount of a gamma Form 2bc T cell antigen receptor polypeptide;
 (b) determining in a sample from a subject the amount of a gamma T cell antigen receptor polypeptide selected from the group consisting of a Form 1 gamma polypeptide, and Form 2abc gamma polypeptide; and
 25 (c) comparing the amount determined in step (a) with the amount determined in step (b), thereby determining the relative usage.

3.1. DEFINITIONS

30 As used herein, the following terms will have the meanings indicated:

TCR = T cell antigen receptor

V = variable

D = diversity

J = joining

35 C = constant

mAb = monoclonal antibody

4. DESCRIPTION OF THE FIGURES

40 Figure 1. Cytofluorographic analysis of T cell lines with anti-TCR δ 1.

Figure 2. Immunochemical analysis of the specificity of mAb anti-TCR δ 1. Surface 125 I-labeled IDP2 cells were immunoprecipitated using control mAb P3 (lanes 1 and 2), anti-leu 4 (lanes 3-5), anti-TCR δ 1 (lanes 6-8), or anti-C γ serum (lane 9) and were then resolved by SDS-PAGE (polyacrylamide gel electrophoresis) and visualized by autoradiography. N = nonreducing conditions; R = reducing conditions.

45 Figure 3. N-glycanase digestion of δ TCR.

Figure 4. Map of pGEM3-0-240/38.

Figure 5. Immunoprecipitation of in vitro translation products of cDNA clone IDP2 0-240/38 by mAb anti-TCR δ 1.

Figure 6. Immunoprecipitation and SDS-PAGE analysis of T cell antigen receptor. Open arrowheads indicate the position of the δ chains. The solid arrowheads indicate the position of the γ chains. Lysates were immunoprecipitated using δ TCAR-3 antibody (odd numbered lanes) or β F1 antibody (even numbered lanes).

Figure 7. Immunoprecipitation of δ chain by δ TCAR-3 antibody. Molt-13 cells solubilized in Tris-buffered saline (pH 8) containing 0.3% CHAPS (lane 1) or in 1% Triton X-100 (lanes 2-7). Lane 1, δ TCAR-3 immunoprecipitates γ , δ TCR heterodimer with the CD3 proteins. Lane 2, δ TCAR-3 immunoprecipitates single δ chain from denatured lysates (N) and reducing (R) conditions, respectively. Lane 5, UCHT-1 immunoprecipitates the CD3 proteins. Lane 6, β F1 antibody does not immunoprecipitate a heterodimer from MOLT-13 cells. Lane 7, anti-C γ antiserum immunoprecipitates a single γ chain.

Figure 8. Analysis of cell surface staining by flow cytometry. γ , δ TCR-positive cells (MOLT-13) PEER, IDP2) and α , β TCR-positive cells (HPB-ALL, Jurkat) were incubated with δ TCAR-3, OKT3, WT31 and normal mouse serum (NMS) antibodies and analyzed by flow cytometry. The B cell line, Daudi, was the negative control.

Figure 9. Two color cytofluorographic analysis of δ TCAR-3⁺ and OKT3⁺ peripheral blood lymphocytes. The fluorescein isothiocyanate (FITC) fluorescence is depicted on the Y axis and phycoerythrin (PE) fluorescence on the X axis. The CD3⁺ γ , δ TCR⁺ cells in this sample represent 2.4% of CD3⁺ lymphocytes.

Figure 10. Measurement of intracytoplasmic Ca²⁺ concentration ([Ca²⁺]_i) versus time. Top panel: δ TCAR-3. Bottom panel: Anti-Leu antibody. Arrows indicate the time of addition of antibody.

Figure 11. Immunoprecipitation of the three forms of γ , δ TCR. For parts A-E, the antibodies used for immunoprecipitation are anti-Leu4 (anti-CD3), β F1 (anti-TCR β), anti- δ 1TCR (anti- δ TCR), anti-Cyb serum (anti- γ TCR) and P3 (unlabelled lanes, control). Immunoprecipitations from ¹²⁵I-labelled cell lysates were analyzed by SDS-PAGE (10% polyacrylamide) under reducing (R) or nonreducing (N) conditions. An open arrow ($\rightarrow$) indicates the position of TCR δ under reducing conditions, whereas the solid arrow ($\blacktriangleright$) denotes the position of δ TCR under nonreducing conditions. Size markers, M_r in thousands, are shown on the left.

A) Nondisulfide-linked γ TCR (40 kD) on PBL-L2. In lanes 1-6 the radiolabelled cells were solubilized in 0.3% CHAPS detergent which preserves the TCR-CD3 association, whereas in lanes 7 and 8, immunoprecipitations were performed after chain separation (see methods).

B) Nondisulfide-linked γ TCR (55 kD) on IDP2 cells. In lanes 1-4 radiolabelled cells were solubilized in 0.3% CHAPS detergent, whereas in lanes 5 and 6 immunoprecipitations were carried out after chain separation.

C) Disulfide-linked γ TCR (40 kD) on WM-14 cells. All lanes correspond to immunoprecipitations from 1% digitonin solubilized radiolabelled cells.

D) Nondisulfide-linked γ TCR (40 kD) on thymic Clone II cells. Radiolabelled cells were solubilized in 1% digitonin (lanes 1-4) or in 0.1% Triton X-100 (lanes 5 and 6), whereas in lanes 7 and 8 immunoprecipitations were carried out after chain separation.

E) Nondisulfide-linked γ TCR (40 kD) on MOLT-13 leukemia T cells. In lanes 1-4 immunoprecipitations were carried out after solubilization of cells in 0.3% CHAPS detergent, whereas in lanes 5 and 6 immunoprecipitations were carried out after chain separation.

Figure 12. Immunoprecipitation of γ TCR and δ TCR chain by anti-Cym1 antibody and anti-TCR δ 1 antibody, respectively. Cell surface radiolabelled MOLT-13 cells were solubilized in 0.3% CHAPS detergent and the γ , δ TCR-CD3 complex was isolated with anti-CD3 monoclonal antibody. Immunoprecipitates were analyzed by 10% SDS-PAGE under reducing conditions.

Lane 1: Immunoprecipitation with anti-Leu4 (anti-CD3) mAb

Lane 3: Immunoprecipitation with anti-Cym1 (anti-TCR γ) mAb after separating chains of isolated γ , δ TCR-CD3 complexes.

Lane 4: Immunoprecipitation with anti-TCR δ 1 (anti-TCR δ) mAb after separating chains of isolated γ , δ TCR-CD3 complexes.

Figure 13. Determination of peptide backbone sizes and glycosylation of γ and δ TCRs from PEER and MOLT-13 cells. Monoclonal antibodies used for immunoprecipitation are anti-Cym1 (anti-TCR γ), anti-TCR δ 1 (anti-TCR δ) and P3 (labelled control) as shown at the top of each lane. The labelled cell lines used are shown at the bottom of each 10% SDS-PAGE autoradiograph or fluorograph. All samples were resolved under reducing conditions. Size markers, M_r in the thousands.

A) Peptide backbone sizes of γ TCR from PEER and MOLT-13 cells. Cells were biosynthetically labelled with ³⁵S-cysteine and ³⁵S-methionine for 15 minutes. Samples were either treated with Endo H (+) or mock treated (-). Immunoprecipitation with anti-Cym1 shows the positions of immature γ TCR of PEER cells (lane 3) and of MOLT-13 cells (lane 7), while the corresponding polypeptide backbone sizes are visualized after treatment with endo H (lanes 4 and 8).

B) Glycosylation of TCR δ from MOLT-13 cells. ¹²⁵I-labelled cells were immunoprecipitated with anti-CD3 mAb and the δ TCR polypeptides were gel purified (see methods) before incubation with N-glycanase (lane 4), endo H (lane 2), or mock treated (lanes 1, and 3).

Figure 14. Nucleotide sequence of MOLT-13 γ TCR (Form 2bc). Part A: Sequencing strategy of clone M13k. A partial restriction map of the 1.1 kb cDNA clone M13k is shown. Part B: Nucleotide and deduced amino acid sequence of clone M13k. Signal sequence (S), variable (V), N-region (N), joining (J) and constant (C1, C1b, C1c and C1d) region gene segments are indicated by arrows and were identified by comparison to genomic sequences, described by Lefranc et al., (1986, Cell 45:237-246) (for S and V), Lefranc et al., (1986, Nature, 319:420-422) and Quertermous et al., (1987, Immunol. 138:2687-2690) (for J) and Lefranc et al., (1986, Proc. Natl. Acad. Sci. U.S.A. 83:9596-9600) and Pellicci et al., (1987, Science 237:1051-1055) (for C). The deduced amino acid sequence beginning at the initiator methionine is presented below the nucleotide sequence. Ex-

tracellular cysteines are highlighted by boxes, and potential N-linked carbohydrate attachment sites (N-X-S or N-X-T; Marshall, 1977, Ann. Rev. Biochem. 41:673-702) are indicated by brackets.

Figure 15. Preferential use of γ,δ TCR Form 1. Freshly isolated peripheral blood mononuclear cells from three healthy donors were ^{125}I -labelled and solubilized in 1% Triton X-100. Immunoprecipitates with P3 (control, lanes 1 and 3), and anti-TCR δ 1 (anti-TCR δ , lanes 2 and 4), were analyzed under nonreducing (N) and reducing (R) conditions. M_r markers in the thousands are shown on the left.

Figure 16. Schematic representation of the three γ,δ TCR forms in man. The CII exon encoded connector peptides are highlighted by filled areas (■) as Cy1 CII exon encoded peptide; (▨), (▩), (▪), as Cy2 CII exon copy a, copy b, and copy c encoded peptides, respectively). Potential N-linked glycan attachment sites (o), and sulfhydryl groups (-SH) and putative disulfide bridges (-S-S-) are indicated.

Figure 17. Map of the rearranged δ TCR gene. A map of r11981 including EcoRI (RI), Hinc II (Hc), ScaI (S) and PvuII (P) sites and probes used in Southern blot analysis is shown.

Figure 18A. Schematic representation of the γ TCR chains used for transfection into MOLT-13 cell line. The schematic is based on reported analyses (Brenner, M.B., et al., 1986, Nature 322:145-149; Brenner, M.B., et al., 1987, Nature 325:689-694; Krangel, M.S., et al., 1987, Science 237:64-67). Pred., predicted; Obs., observed. The predicted glycosylated polypeptide size assumes that all available N-linked glycosylation sites (shown as lollipops), each containing 3 kD of attached carbohydrate, are used, and that no significant size differences are introduced by other post-translational modifications. The intra-chain disulfide linkages typical of Ig-like molecules are shown. Note that a cysteine residue (cys) is encoded by the CII exon in PBL C1 γ TCR, but such a cysteine is absent from all the copies of CII exon used in the two other γ TCR chains. γ TCR constant region in the γ,δ T leukemia cell line PEER (Littman, D.R., et al., 1987, Nature 326:85-88) that also expresses a 55 kD γ TCR protein (Brenner, M.B., et al., 1987, Nature 325:689-694; Weiss, A, et al., 1986, Proc. Natl. Acad. Sci. USA 83:6998-7002) is identical to that of IDP2.

Figure 18B. The expression plasmid constructs pFneo.PBL C1 γ and pFneo.IDP2 γ were used to introduce γ TCR clones into the MOLT-13 cell line. PBL C1 γ TCR cDNA clone (PBL C1.15) and repaired IDP2 γ TCR cDNA clone (IDP2.11r) (Krangel, M.S., et al., 1987, Science 237:64-67) were cleaved from their parent plasmid vector (pUC 18) by EcoRI digestion, the ends were made blunt with Klenow fragment of DNA polymerase I, and the cDNAs were then ligated into a Sall-cut, and Klenow-treated pFneo mammalian expression vector. Clones containing the cDNA inserts in appropriate orientation with respect to the spleen focus forming virus (SFFV) LTR were selected based on restriction mapping. pFneo (Saito, T., et al., 1987, Nature 325:125-130) is a derivative of pT β Fneo (Ohashi, P., et al., 1985, Nature 316:606-609) obtained by BamHI digestion, to delete the murine β TCR cDNA insert, followed by ligation with T4 DNA ligase. As shown, this vector contains a bacterial neomycin resistance gene (neo^r) under the control of SV40 promoter, thus conferring resistance to the antibiotic G418 on the mammalian recipient cells. The restriction sites within parentheses were destroyed during construction.

Figure 19. Immunoprecipitation analysis of γ,δ TCR on Molt-13 γ TCR transfectants. Surface ^{125}I -labeled cells were solubilized in 0.3% CHAPS detergent to preserve the chain association, immunoprecipitated with mAb P3 (control), anti-leu-4 (anti-CD3), anti-TCR δ 1 (anti- δ TCR), or anti-Ti- γ A (anti-V γ 2), and were then resolved by SDS-PAGE under nonreducing (N) or reducing (R) conditions and visualized by autoradiography as described earlier (Brenner, M.B., et al., 1986, Nature 322:145-149; Brenner, M.B., et al., 1987, Nature 325:689-694). Anti-Ti- γ A mAb shows a pattern of reactivity on different T cell clones consistent with its recognition of V γ 2 segment. M13.PBL C1 γ : MOLT-13 cells transfected with the PBL C1-derived γ TCR cDNA; Clone #7 was used for this analysis. M13.IDP2 γ : MOLT-13 cells transfected with the IDP2-derived γ TCR cDNA; Clone #10 was used for this analysis. Size markers, M_r (molecular weight) in thousands of daltons. Open arrow, resident MOLT-13 γ TCR chain; solid arrow, transfected (PBL C1- or IDP2-derived) γ TCR cDNA; asterisk, MOLT-13 δ TCR chain under nonreducing conditions. Upon reduction, the δ TCR chain undergoes a mobility shift and comigrates with the 40 kD γ TCR chain. However, δ TCR chain is distinctly visualized as a 40 kD band under reducing conditions when the 40 kD γ TCR protein is not coimmunoprecipitated, as is seen in anti-V γ 2 immunoprecipitates of M13.IDP2 γ (Fig. 19, lane 8).

Figure 20. Two-dimensional gel analysis of γ TCR polypeptides of transfectants. 2×10^7 cells were surface ^{125}I labeled, treated with neuraminidase (150 units in 1.5 ml PBS with 1 mg/ml each of glucose and bovine serum albumin for 1.5 hours at 23°C), solubilized in 0.3% CHAPS detergent, immunoprecipitated with 1 μg anti-leu-4 mAb, and subjected to 2D gel analysis under reducing conditions. Non-equilibrium pH gradient gel electrophoresis (NEPHGE) was carried out using pH 3.5 to 10 Ampholines (LKB, Sweden) followed by SDS-polyacrylamide gel electrophoresis on a 10.5% acrylamide gel (Brenner, M.B., et al., 1987, Nature 325:689-694). Positions of the CD3 components (not shown) were used to identify and compare the γ TCR species expressed in different cell lines. M13.PBL C1 γ transfectant clone #10 and M13.IDP2 γ transfectant clone #10 were used. Open arrows, MOLT-13 γ TCR species; solid arrows, IDP2 γ TCR species; asterisk, PBL C1 γ TCR species.

Figure 21. Analysis of backbone polypeptide sizes of γ TCR chains of transfectants. Cells were pulse labeled with ^{35}S -methionine and ^{35}S -cysteine for 15 minutes and immunoprecipitated with P3 (control), anti-C γ m1 (anti- γ TCR), or anti-TI- γ A (anti-V γ 2) mAb, as indicated. Immunoprecipitates were treated with endoglycosidase-H (Endo-H, +), or were mock-incubated (-), resolved by SDS-PAGE, and visualized by fluorography. All samples were run reduced. M $_r$, molecular weight markers in thousands of daltons. The 43 kD contaminating actin band serves as an additional internal marker. M13.IDP2 γ : MOLT-13 cells transfected with the IDP2 γ TCR cDNA, clone #10; M13.PBL C γ : MOLT-13 cells transfected with the PBL C γ γ TCR cDNA, clone #10.

Figure 22. Southern blot analysis of γ 6 T cell clones and polyclonal human T cell populations. Genomic DNA was digested with *Eco*RI and probed with the V-J probe. DNA sources are: PBL T-cell clones, (lanes 1, 3-9), PBL (lane 10), newborn thymocytes (NBT-lane 11), fetal thymocytes (FT-lane 12), and B cells (germline-lane 2). The germline 3 kb V δ and 6.7 kb J δ fragment are indicated on the left of the blot, while the 5 common rearrangements, numbered I-V are indicated on the right. The sizes of the rearrangements from I-V are 2.9 kb, 3.5 kb, 4.2 kb, 6.2 kb and 7.1 kb respectively.

5. DETAILED DESCRIPTION OF THE INVENTION

5.1. THE γ TCR FORM 2bc POLYPEPTIDE AND NUCLEIC ACIDS

The invention is directed to a form of the human γ TCR polypeptide termed Form 2bc (detailed *infra* in Sections 8 and 9). The invention is also directed to nucleic acids encoding γ Form 2bc, such as DNA and RNA, and their complementary nucleic acids.

Form 1 and Form 2abc γ TCR polypeptides are previously reported forms of the human γ TCR (see PCT International Publication No. WO 88/00209, Published January 14, 1988). The Form 1 γ TCR polypeptide has a molecular weight of about 40,000 daltons. The Form 2abc γ TCR polypeptide has a molecular weight of about 55,000 daltons. Form 2abc γ TCR chain has a slightly larger peptide backbone and contains one extra potential N-linked glycan than Form 1. In contrast, the γ TCR chain of the invention, Form 2bc, has a molecular weight of about 40,000 daltons. Furthermore, the Form 2bc γ TCR polypeptide possesses a slightly smaller peptide backbone and 2-3 less N-linked glycans.

γ TCR chain Form 2bc differs in size by more than 15 kD (40 kD versus 55 kD) compared to the previously described Form 2abc. This difference is accounted for by a 5 kD smaller polypeptide backbone size (35 kD versus 40 kD) and by a reduction in the amount of carbohydrates (5 kD versus 15 kD). The approximately 35 kD polypeptide backbone size of Form 2bc also serves to distinguish it from Form 1; Form 1 has a 40 kD backbone size.

γ TCR polypeptide Form 2bc also differs from Form 1 and Form 2abc in constant region (C γ) gene segment usage. Form 1 γ TCR chains have a constant region encoded by the C γ 1 gene segment (Krangel et al., 1987, Science 237:64-67) containing a single CII exon. The Form 2abc γ polypeptide is encoded by C γ 2 gene segments containing three CII exon copies, namely copy a, copy b and copy c (Krangel et al., 1987, Science 237:64-67; Littman et al., 1987, Nature 326:85-88). In contrast, Form 2bc lacks one copy of the sequence encoded by the C γ 2 second exon that is present in the cDNA of Form 2abc. This, Form 2bc contains two C γ 2 CII exon copies, namely copy b and copy c. Copy a of CII, which is missing in Form 2bc, encodes a part of a connector region between the membrane spanning region and the extracellular constant domain.

Six potential N-linked carbohydrate attachment sites exist on the Form 2bc polypeptide. Since the biochemical data suggest that only 2-3 N-linked glycans are attached to the polypeptide chain, it indicates that not all potential sites are used.

In specific embodiments, γ TCR polypeptide Form 2bc can be obtained from cells of the MOLT-13 (Loh et al., 1987, Nature 330:569-572) T cell line or thymus-derived Clone II (Bank et al., 1986, Nature 322:179-181). γ TCR chain Form 2bc can also be obtained from T lymphocytes of a human subject which express that γ TCR form.

Form 2bc γ TCR polypeptide comprises the primary amino acid sequence of the γ TCR polypeptide shown in Figure 14, or any portion thereof comprising a constant region consisting of copy b and copy c of C γ 2 CII.

The present invention also provides a nucleic acid molecule encoding a γ TCR Form 2bc polypeptide having a molecular weight of about 40,000 daltons. The constant region of γ TCR Form 2bc polypeptide results from translation of a nucleic acid sequence which has only two of the three C γ 2 cII exons. The invention is also directed to nucleic acid sequences comprising a C γ 2 constant region having only two cII exons. The nucleic acid can be a DNA, cDNA, RNA, and complementary nucleic acids and derivatives thereof. In a specific embodiment of the invention, the DNA molecule comprises at least a portion of the nucleic acid sequence shown in Figure 14.

In an example to be discussed in Section 8, the 2bc γ TCR polypeptide and its encoding nucleic acid se-

quence are described. In an example to be discussed in Section 9, it is shown that the ability of the γ TCR polypeptide to form disulfide bonds or be glycosylated is determined by its constant region primary sequence.

5.2. POLYPEPTIDE COMPLEXES CONTAINING γ TCR FORM 2bc

The present invention also relates to polypeptide complexes which comprise the γ TCR chain Form 2bc. In a specific embodiment, the polypeptide complex consists of a T cell antigen receptor dimer. In particular, such a dimer can be a heterodimer (including but not limited to a γ,δ heterodimer, a γ,β heterodimer, and a α,γ heterodimer, or a γ,γ' heterodimer in which γ' can be γ TCR polypeptide Form 1, 2abc, or 2bc), or a homodimer.

In a particular embodiment of the invention, the polypeptide complex comprising γ TCR Form 2bc is a $\gamma\delta$ TCR heterodimer. Thus, a purified complex which comprises at least a portion of a δ TCR polypeptide and γ TCR Form 2bc polypeptide is provided by the present invention. The δ polypeptide may have at least one intrachain, covalent, disulphide bridge. Additionally, the polypeptide may comprise a δ TCR polypeptide having a molecular weight of about 40,000 daltons.

As detailed in the examples *infra*, the γ TCR Form 2bc chain is noncovalently associated in a complex with the δ TCR chain. Thus, γ Form 2bc forms a nondisulfide-linked TCR complex. γ TCR chain Form 2abc also forms a nondisulfide-linked complex with a δ TCR chain (e.g., on IDP2 cells), while γ TCR chain Form 1 forms a disulfide-linked complex with a δ TCR polypeptide.

As shown in the example of Section 9, *infra*, γ TCR constant region CII exon usage (and thus the primary sequence of the γ TCR chain) determines not only the presence or absence of disulfide linkage between TCR γ and δ , but also the amount of carbohydrate attached to γ TCR, which is largely responsible for the differences in size of the cell surface γ TCR proteins. Thus, the present invention also provides a method for producing expression of $\gamma\delta$ TCR heterodimers of defined intermolecular linkage (disulfide or nondisulfide-linked) and extent of γ TCR glycosylation, which comprises introducing a γ TCR gene encoding a particular γ polypeptide form into a cell capable of expressing the γ gene, which cell expresses the δ TCR chain.

The present invention further provides a purified complex which comprises a γ TCR Form 2bc polypeptide of the present invention associated with another γ TCR polypeptide (e.g., Form 1, 2abc, or 2bc). In one embodiment of the invention, the two γ TCR polypeptides are associated with each other through at least one interchain, covalent, disulfide linkage. In another embodiment of the invention, the two γ TCR polypeptides are noncovalently associated with each other. In still another embodiment of the invention, the two γ TCR polypeptides have the same constant domain. In yet a further embodiment of the invention, the two γ TCR polypeptides have different constant domains.

5.3. MONOCLONAL ANTIBODIES REACTIVE WITH THE $\gamma\delta$ TCR POLYPEPTIDES

A monoclonal antibody (mAb) to an epitope of the γ or δ T cell antigen receptor can be prepared by using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein (1975, Nature 256:495-497), and the more recent human B cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72) and EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

In one embodiment, the monoclonal antibodies may be human monoclonal antibodies or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, Proc. Natl. Acad. Sci. U.S.A. 80:7308-7312; Kozbor et al., 1983, Immunology Today 4:72-79; Olsson et al., 1982, Meth. Enzymol. 92:3-16). Chimeric antibody molecules may be prepared containing a mouse (or rat, or other species) antigen-binding domain with human constant regions (Morrison et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851; Takeda et al., 1985, Nature 314:452).

The invention is also directed to a method of identifying a monoclonal antibody reactive with a T cell antigen receptor. Such a mAb can be identified by detecting its ability to comodulate the CD3 antigen upon binding of the mAb to a cell which expresses both a T cell antigen receptor and CD3 complex. The CD3 comodulation can be detected, for example, by measuring the amount of labeled anti-CD3 antibody which is bound by the cell. This method is illustrated by way of example in Section 7.1.1, *infra*, in which it is used to identify hybridomas secreting anti- V_δ mAb δ TCAR-3.

A molecular clone of an antibody to an epitope of a γ or δ TCR polypeptide can be prepared by known techniques. Recombinant DNA methodology (see e.g., Maniatis et al., 1982, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York) may be used to construct nucleic acid sequences which encode a monoclonal antibody molecule, or antigen binding region thereof.

Antibody molecules may be purified by known techniques, e.g., immunoabsorption or immunoaffinity chro-

matography, chromatographic methods such as HPLC (high performance liquid chromatography), or a combination thereof, etc.

Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment which can be produced by pepsin digestion of the antibody molecule; the Fab' fragments which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragment, and the Fab fragments which can be generated by treating the antibody molecule with papain and a reducing agent.

One embodiment of the invention is directed to monoclonal antibodies reactive with the variable region of the δ TCR chain. Such an antibody is δ TCAR-3 (aka TCS δ 1) (see Section 7, *infra*), which recognizes an epitope expressed from a specific δ gene rearrangement. As described in Section 7.2, *infra*, mAb δ TCAR-3 is capable of stimulating the proliferation of a γ, δ^+ T lymphocyte. Monoclonal antibody δ TCAR-3 is also able to stimulate a rise in cytoplasmic free calcium ion concentration of γ, δ^+ T lymphocytes.

In another embodiment, the invention relates to antibodies reactive with the constant region of the γ or δ TCR polypeptide. In a specific embodiment, the invention is directed to mAb TCR δ 1 which is reactive with the constant region of the δ TCR chain (see Section 6, *infra*). In another specific embodiment, the invention relates to mAb anti-C γ m1, which is reactive with the constant region of the γ TCR chain (see Section 8.1.7, *infra*).

6. GENERATION OF MONOCLONAL ANTIBODY ANTI-TCR δ 1 SPECIFICALLY REACTIVE WITH THE TCR DELTA SUBUNIT CONSTANT REGION

6.1. EXPERIMENTAL PROCEDURES

6.1.1. CYTOFLUOROGRAPHIC ANALYSIS OF T CELL LINES WITH ANTI-TCR δ 1

The anti-TCR δ 1 mAb, which is specifically reactive with the δ TCR chain constant region, was made as follows: One gram of PEER cells were solubilized in 50 ml of 0.3% CHAPS (3-[3-cholamidopropyl]dimethylammonio]1-propanesulfonate) detergent and were immunoprecipitated with 1 μ l of UCHT1 (Beverly, P.C., and Callard, 1981, Eur. J. Immunol. 11:329-334) ascites, 500 μ l of mAb 187.1 culture supernatant and Staphylococcus aureus Cowan I strain (SACI). Four intraperitoneal injections at six week intervals were carried out, followed by a final boost of $\gamma\delta$ TCR (without CD3) isolated by selective elution of $\gamma\delta$ TCR from the immune complexes using 2% Triton X-100. The eluted material was administered both intravenously and intraperitoneally; four days after this boost, the mice were sacrificed and fusion carried out as previously described (Brenner, M.B., et al., 1987, J. Immunol. 138:1502-1509).

$\gamma\delta$ TCR cell lines PEER and IDP2 or $\alpha\beta$ TCR cell lines HPB-MLT and JURKAT were stained with 50 μ l of anti-TCR δ 1 culture supernatant followed by staining with FITC-conjugated goat anti-mouse Ig F(ab') $_2$ fragments with analysis on an Ortho cytofluorograph (see Fig. 1). Control was the mAb secreted by P3X63.Ag8 hybridoma (P3) and anti-CD3 mAb was anti-Leu 4 (Ledbetter, J.A., et al., 1981, J. Exp. Med. 153:310).

6.1.2. IMMUNOCHEMICAL ANALYSIS OF THE SPECIFICITY OF mAb anti-TCR δ 1

Surface 125 I-labeled IDP2 cells were solubilized and their proteins immunoprecipitated using control mAb P3, anti-Leu 4, anti-TCR δ 1, or anti-C γ serum. Precipitated samples were analyzed by SDS-PAGE followed by autoradiography. In CHAPS detergent, $\gamma\delta$ TCR and CD3 remained associated and were immunoprecipitated as a complex by anti-Leu 4 (Fig. 2, lanes 3 and 4). However, after solubilization in 2% Triton X-100 detergent, anti-TCR δ 1 immunoprecipitated $\gamma\delta$ TCR as a dimeric complex without CD3 (lane 6) and anti-Leu 4 immunoprecipitated CD3 as a trimeric complex without $\gamma\delta$ TCR (lane 5). After separation of the $\gamma\delta$ TCR-CD3 component chains, anti-TCR δ 1 immunoprecipitated TCR δ alone (lane 7 and 8), while anti-C γ serum immunoprecipitated TCR γ alone (lane 9). For chain separation experiments (lane 7-9), anti-Leu 4 immunoprecipitates from CHAPS solubilized IDP2 cells were boiled in 1% SDS and were then diluted with 4 volumes of 2% Triton X-100 followed by immunoprecipitation with anti-TCR δ 1 or anti-C γ serum. This follows procedures used previously (Brenner, M.B., et al., 1986, Nature 322:145).

6.1.3. N-LINKED GLYCOSYLATION OF THE TCR δ POLYPEPTIDE

125 I-labeled IDP2 cells were solubilized in 0.3% CHAPS, immunoprecipitated with anti-Leu 4 and resolved by SDS-PAGE (Fig. 3). Control lane is mock-digested IDP2 δ TCR polypeptide. N-glycanase digestion of δ TCR polypeptide was performed as follows: δ TCR was eluted from a gel slice followed by N-glycanase (Genzyme Corp.) digestion (10 U/ml) carried out in 30 μ l 0.17% SDS, 1.25% Nonidet P-40, 0.2 M sodium phosphate buffer

pH 8.6 for 16 hours at 37°C (Tarentino, A.L., et al., 1985, *Biochemistry* 24:4665). The digested or mock-incubated δ TCR samples were analyzed by SDS-PAGE and visualized by autoradiography.

6.1.4. RECOGNITION OF IN VITRO TRANSLATION PRODUCTS OF cDNA CLONE IDP20-240/38 BY mAb ANTI-TCR δ 1

A plasmid designated pGEM3-O-240/38 was constructed as follows and used for *in vitro* transcription-translation (Fig. 4). The IDP2 O-240/38 (δ TCR) cDNA clone 1.5 kb insert begins within codon 7 of the composite Group O sequence and includes the remaining coding region and most of the 3' untranslated region. This insert was cleaved as a single EcoRI fragment from λ gt10 arms by partial EcoRI digestion (to prevent cleavage of the internal EcoRI site). This fragment was subcloned into a Bluescript+ vector (Stratagene). The insert was then removed from the vector as a single BamHI-SalI fragment (ends are from the Bluescript vector polylinker) facilitating directional cloning into pGEM-3 (Promega Biotech) downstream of the T7 promoter. The resultant pGEM3-O-240/38 plasmid was linearized with SalI and capped transcripts synthesized using T7 RNA polymerase (Krangel, M.S., et al., 1987, *Science* 237:64). Integrity and size of the transcripts were monitored via an aliquot of the reaction mixture containing 32 P-ATP. A single RNA species of 1.5 kb was observed. *In vitro* translation in the presence of 35 S-methionine was performed in a rabbit reticulocyte lysate. After *in vitro* translation, the samples were boiled in 1% SDS with 2 mM dithiothreitol followed by the addition of 10 volumes of 2% Triton X-100 in Tris buffered saline pH 7.5. Samples were immunoprecipitated with control mAb P3 (Fig. 5, lanes 1 and 3) or with anti-TCR δ 1 mAb (lanes 2 and 4) and analyzed by SDS-PAGE followed by fluorography (Bonner, W.J. and Laskey, R.A., 1974, *Eur. J. Biochem.* 46:83-88).

6.2. EXPERIMENTAL RESULTS

We have generated a monoclonal antibody (mAb), anti-TCR δ 1, that is specifically reactive with the δ TCR constant region.

The $\gamma\delta$ TCR-CD3 complex from the PEER cell line (Weiss, A., et al., 1986, *Proc. Natl. Acad. Sci. U.S.A.* 83:6998-7002; Brenner, M.B., et al., 1987, *Nature* 325:689-694) was used as immunogen in the production of antibody-secreting hybridoma cell lines. Hybridomas were screened both by cell surface binding (cytofluorographic analysis) and by immunoprecipitation of PEER cell proteins followed by SDS-PAGE analysis. 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 $\gamma\delta$ TCR lymphocytes (PEER, IDP2) but failed to react with $\alpha\beta$ TCR cells (HPB-MLT, JURKAT) or with non-T leukocytes (Fig. 1 and data not shown). Although the immunogen was composed of a complex of $\gamma\delta$ TCR and CD3, the greater affinity of the mAb for $\gamma\delta$ TCR cell lines suggested the mAb was not directed against CD3 determinants.

The specificity of the mAb was determined in immunoprecipitation studies using various detergents which affect the association of the proteins comprising the receptor complex. After 125 I-labeled IDP2 cells were solubilized in CHAPS detergent, TCR γ and δ , and CD3 γ , δ , and ϵ subunits remained part of an associated complex immunoprecipitated by anti-CD3 antibody (Fig. 2, lanes 3, 4). However, if radiolabeled IDP2 cells were solubilized in 2% Triton X-100 detergent, $\gamma\delta$ TCR and CD3 became largely dissociated, and the use of anti-CD3 mAb resulted in selective precipitation of CD3 (Fig. 2, lane 5). Under these latter conditions, mAb 5A6.E9 immunoprecipitated $\gamma\delta$ TCR as a heterodimer without associated CD3 (Fig. 2, lane 6). This observation provided the first direct evidence that TCR γ and TCR δ exist as a non-disulfide-linked heterodimer. To determine whether mAb 5A6.E9 reacts with a γ TCR chain, δ TCR chain or a combinatorial determinant, immunoprecipitation of separated polypeptide chains was performed. An anti-Leu 4 immunoprecipitate from radiolabeled, CHAPS-solubilized IDP2 cells was boiled in 1% SDS to dissociate the γ TCR, δ TCR, and CD3 proteins. After dilution with four volumes of 2% Triton X-100, mAb 5A6.E9 specifically immunoprecipitated the 40 kD (δ TCR) species (Fig. 2, lane 7). When an aliquot of the same immunoprecipitate was analyzed under reducing conditions (Fig. 2, lane 8), a dramatic shift in SDS-PAGE mobility was observed. This phenomenon is characteristic of δ TCR from the IDP2 and PEER cell lines (Brenner, M.B., et al., 1987, *Nature* 325: 689-694). In contrast, when the separated chains were immunoprecipitated with the anti-C γ sera, the 55 kD species (γ TCR), but not the 40 kD species (δ TCR) was immunoprecipitated (Fig. 2, lane 9). Based on these biochemical and surface binding studies, mAb 5A6.E9 is referred to as anti-TCR δ 1.

In addition to PEER and IDP2, anti-TCR δ 1 also immunoprecipitated TCR δ from other $\gamma\delta$ TCR cell lines including MOLT-13 and PBL line 2. Further experiments have shown that anti-TCR δ 1 reacts with a determinant encoded by a TCR δ constant (C) gene segment.

We have isolated cDNA clones from the IDP2 cell line (e.g., IDP2 O-240/38) by the subtractive approach representing a gene which encodes the TCR δ subunit. Genes to which IDP2 group O cDNA clones hybridize

in Southern blotting experiments are expressed and rearranged in $\gamma\delta$ TCR lymphocytes but are typically not expressed (and are often deleted) in $\alpha\beta$ TCR cells. By sequence comparison with other TCR genes, these cDNA clones appear to be composed of novel V, D (?), J, and C gene segments. The IDP2 Group O composite DNA sequence contains a long open reading frame predicting a polypeptide with two potential asparagine-linked glycosylation sites and a molecular weight of 31.3 kilodaltons. To determine the molecular weight of the unglycosylated δ TCR protein and the number of asparagine-linked carbohydrates that are present on the mature IDP2 δ TCR polypeptide, gel purified δ TCR was either treated with N-glycanase or mock-incubated and analyzed by SDS-PAGE (Fig. 3). Removal of N-linked carbohydrates resulted in a 5 kD decrease in apparent molecular weight (40 kD to 35 kD), suggesting the presence of two (2.5-3 kD) N-linked glycans on the IDP2 δ TCR. This correlates well with the number of N-linked glycans predicted by the translated amino acid sequence in Figure 5. The apparent molecular weight of the protein is in general agreement, differing from that predicted by 3.7 kD.

Given the reactivity of anti-TCR δ 1 on IDP2 cells, the specificity for the δ TCR polypeptide, and the recognition of partially denatured (SDS boiled) δ TCR, we tested whether this mAb would recognize directly polypeptide encoded by the δ TCR cDNA clone. Thus, the insert from cDNA clone IDP2 O-240/38 was subcloned into the pGEM-3 expression vector downstream of the T7 promoter (Fig. 4). Transcripts generated *in vitro* with T7 RNA polymerase were then used in a rabbit reticulocyte lysate system to direct the synthesis of protein in the presence of 35 S-methionine. Following *in vitro* transcription-translation, the reaction mixtures were boiled in 1% SDS, diluted with ten volumes of 2% Triton X-100, and then immunoprecipitated with either an isotype-matched control mAb or with anti-TCR δ 1. Anti-TCR δ 1 mAb specifically immunoprecipitated a predominant species (34 kD) (Fig. 5, lane 4). No such band was observed in immunoprecipitates when control mAbs were used (lane 3), when RNA transcripts were omitted (lanes 1 and 2), or when γ TCR constructs were used. Thus, the radiolabeled species immunoprecipitated by mAb anti-TCR δ 1 corresponds to a δ polypeptide whose synthesis was specifically directed by the IDP2 O-240/38 cDNA clone. This polypeptide (34 kD) is very similar in size to the N-glycanase treated IDP2 δ TCR chain (35 kD). The IDP2 O-240/38 clone lacks a natural ATG initiation codon as well as the leader sequence. There are two potential internal ATG codons (at residues 12 and 44) within the V region of this clone (Fig. 5). Use of these codons to initiate synthesis could result in more than one polypeptide species possibly accounting for the minor species noted (Fig. 5, lane 4). Thus, there is direct serological recognition by mAb anti-TCR δ 1 of the IDP2 δ TCR subunit encoded by clone IDP2 O-240/38.

7. GENERATION OF MONOCLONAL ANTIBODY δ TCAR-3 SPECIFICALLY REACTIVE WITH THE TCR DELTA SUBUNIT VARIABLE REGION

7.1. EXPERIMENTAL PROCEDURES

7.1.1. IMMUNOPRECIPITATION AND SDS-PAGE ANALYSIS OF T CELL ANTIGEN RECEPTOR

The δ TCAR-3 mAb, specifically reactive with the variable region of the δ TCR chain, was generated as follows: One mouse was immunized with 2×10^7 Molt-13 cells by intraperitoneal injection. One month later, the mouse was boosted with 1×10^7 Molt-13 cells by intravenous injection each day for 3 sequential days, and then immune splenocytes were fused with mouse myeloma P3x63Ag8.653 cells in the presence of 50% polyethylene glycol 1500. The hybridomas were screened by analyzing the CD3 co-modulation with flow cytometry. The analysis of CD3 co-modulation was based on the observation that antibody to $\alpha\beta$ T cell antigen receptor, when incubated with the cells, caused the internalization of the CD3 complex (Lanier, L.L., et al., 1986, J. Immunol. 137:2286; Meuer, S.C., et al., 1983, J. Exp. Med. 157:705).

Molt-13, PEER, and HPB-ALL cell lines were iodinated using the lactoperoxidase technique. The 125 I- β F1 labeled cells were solubilized in Tris-buffered saline (pH 8) containing 1% Triton X-100. Lysates were immunoprecipitated using δ TCAR-3 antibody or β F1 antibody. β F1 is a framework monoclonal antibody to the β TCR chain and is described elsewhere (Brenner, M.B., et al., 1987, J. Immunol. 138:1502-1509). All samples were analyzed by SDS-PAGE under reducing or non-reducing conditions (Fig. 6). Molt-13 and PEER are both CD3 $^{+}$ 4 $^{-}$ 8 $^{-}$ WT31 $^{-}$. HPB is CD3 $^{+}$ 4 $^{+}$ 8 $^{+}$ WT31 $^{+}$.

As shown in Figure 6, δ TCAR-3 immunoprecipitated non-disulfide-linked γ and δ chains from Molt-13 and PEER cells, while β F1 immunoprecipitated disulfide-linked α and β chains from HPB-ALL cells. The difference in autoradiographic intensity between the bands corresponding to the δ and γ chains represent differences in the extent of iodination of these two proteins.

7.1.2. IMMUNOPRECIPITATION OF δ TCR CHAIN BY δ TCAR-3 ANTIBODY

Figure 7 shows ^{125}I -labeled Molt-13 cells solubilized in Tris-buffered saline (pH 8) containing 0.3% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]1-propanesulfonate) or in 1% Triton X-100. In 1% Triton X-100, the $\gamma\delta$ TCR dissociates from the CD3 complex, while in 0.3% CHAPS, the $\gamma\delta$ TCR remains associated with the CD3 complex. Prior to immunoprecipitation, the ^{125}I -labeled lysates used in lanes 3, 4, and 7 of Figure 7 were denatured by adding SDS to a final concentration of 1% followed by heating for 5 minutes at 68°C . After cooling, iodoacetamide was added to a final concentration of 20 mM. The mixture was then diluted with 4 volumes of 1.5% Triton X-100 in Tris-buffered saline (pH 8). This denaturing process completely dissociates γ chain, δ chain, and CD3 proteins from one another. All samples were analyzed by SDS-PAGE under non-reducing conditions (N) except for the sample in lane 4 which is under reducing conditions (R). Note the difference in mobility of δ chain under reducing and non-reducing conditions. The anti-C γ antiserum was generated by immunizing a rabbit with a 20 amino acid synthetic peptide from the γ constant region (residues 117-136).

7.1.3. ANALYSIS OF CELL SURFACE STAINING BY FLOW CYTOMETRY

5×10^5 cells were incubated with the appropriate antibodies (NMS (normal mouse serum), δ TCAR-3, OKT3, or WT31) at 4°C for 30 minutes and then washed two times with 0.2% BSA in PBS (pH 7.4). Following incubation with fluorescein-conjugated goat anti-mouse IgG for 30 minutes at 4°C , cells were analyzed on an Ortho cytofluorograph (Fig. 8).

7.1.4. TWO COLOR CYTOFLUOROGRAPHIC ANALYSIS OF δ TCAR-3 $^+$ AND OKT3 $^+$ PERIPHERAL BLOOD LYMPHOCYTES

The peripheral blood lymphocytes were first incubated with δ TCAR-3 at 4°C for 30 minutes. After washing, cells were incubated with phycoerythrin (PE)-conjugated goat anti-mouse IgG for an additional 30 minutes at 4°C . After washing, the cells were incubated with fluorescein (FITC)-conjugated OKT3 for 30 minutes at 4°C and then cells were analyzed on an Ortho cytofluorograph (Fig. 9).

7.1.5. MEASUREMENT OF INTRACYTOPLASMIC Ca^{2+} CONCENTRATION ($[\text{Ca}^{2+}]_i$) VERSUS TIME

Molt-13 cells were labeled with the acetoxymethyl ester form of the Ca^{2+} -sensitive probe fura-2 ($2 \mu\text{M}$ from a 1 mM stock in dimethyl sulfoxide, Molecular Probes, Eugene, Oregon) at a concentration of 10^7 cells/ml in RPMI 1640 plus 10% fetal bovine serum for 30 minutes at 37°C . Cells were then washed and resuspended at 10^7 cells/ml in Hanks balanced salt solution (HBSS) plus 5% fetal bovine serum and kept in the dark at room temperature until use. Immediately prior to fluorescent measurement, 2×10^6 cells were centrifuged then resuspended in 2 ml of fresh HBSS and placed in a quartz cuvette at 37°C and constantly stirred. Fluorescence was measured on the cell suspension in a SPF-500C fluorometer (SLM Aminco, Urbana, Illinois), the excitation wavelength alternating between $340 (\pm 2)$ and $380 (\pm 2)$ nm and emission was detected at $510 (\pm 5)$ nm. The ratio of 350/380 was automatically calculated (1 ratio every 2 seconds), plotted, and stored in an IBM PC AT. Quantitation of $[\text{Ca}^{2+}]_i$ from the fluorescence ratio was performed as described by Grynkiewicz, et al. (1985), J. Biol. Chem. 260:3440). Addition of irrelevant antibodies did not alter $[\text{Ca}^{2+}]_i$, while cell lysis resulted in $[\text{Ca}^{2+}]_i$ of $1 \mu\text{M}$.

7.2. RESULTS

We have generated a monoclonal antibody, δ TCAR-3, that is directed against a variable region of the TCR δ chain and which can be used to characterize the δ polypeptide. This monoclonal antibody binds to T cells bearing the $\gamma\delta$ TCR and also elicits a fura-2 Ca^{2+} signal upon binding to Molt-13 cells.

The δ TCAR-3 monoclonal antibody was generated by immunizing a mouse with the Molt-13 cell line which has a $\text{CD3}^+4^-8^- \text{WT31}^-$ phenotype. The hybridomas were first screened by CD3 co-modulation. The positive clones were further screened by immunoprecipitation. δ TCAR-3 immunoprecipitation of $\gamma\delta$ TCR heterodimer from ^{125}I -labeled Molt-13 and PEER lysates is shown in Figure 6. δ TCAR-3 does not immunoprecipitate any polypeptide from HPB-ALL (Fig. 6). In contrast, βF1 , a framework monoclonal antibody specific to the β chain (Brenner, M.B., et al., 1987, J. Immunol. 138:1502-1509), immunoprecipitates the $\alpha\beta$ heterodimer from the HPB-ALL cell line (Fig. 6, lanes 10 and 12). The immunoprecipitated $\gamma\delta$ receptor from both Molt-13 and PEER cells, when analyzed under either reducing or non-reducing conditions, displays a heterodimeric structure indicating a non-disulfide-linked $\gamma\delta$ TCR in these two cell lines. There is a slight shift in mobility of the δ chain

under reducing conditions relative to that observed under non-reducing conditions (Fig. 6, lanes 1 and 3, 5 and 7), a phenomenon which has been noted previously in IDP2 and PEER cell lines (Brenner, M.B., et al., 1987, Nature 325:689-694), suggesting the existence of intrachain disulfide linkages. In order to demonstrate that the δ TCAR-3 antibody recognizes a CD3-associated $\gamma\delta$ TCR, immunoprecipitations were performed using ^{125}I -labeled Molt-13 cell lysates solubilized in 0.3% CHAPS detergent (Fig. 7, lane 1). Under these conditions, the CD3 complex remains associated with the receptor, and both $\gamma\delta$ heterodimer and the CD3 complex are immunoprecipitated by δ TCAR-3. However, when ^{125}I -labeled lysates were solubilized in 1% Triton X-100 detergent which largely dissociates the CD3 complex from the $\gamma\delta$ receptor, only $\gamma\delta$ heterodimer is immunoprecipitated by δ TCAR-3 (Fig. 7, lane 2). As a control, the anti-CD3 antibody, UCHT-1 (Beverley, P.C. and Callard, R.E., 1981, Eur. J. Immunol. 11:329-334) immunoprecipitates only the CD3 complex, but not the $\gamma\delta$ heterodimer (Fig. 7, lane 5).

The specificity of δ TCAR-3 was further analyzed by using immunoprecipitations of denatured, ^{125}I -labeled Molt-13 lysates in which $\gamma\delta$ TCR and CD3 proteins were completely dissociated. δ TCAR-3 specifically immunoprecipitated the δ chain which has an apparent molecular weight of 38 kD under non-reducing conditions (Fig. 7, lane 3) and 40 kD under reducing conditions (lane 4). The anti-C γ antiserum immunoprecipitated the γ chain with molecular weight 42 kD under reducing conditions (Fig. 7, lane 7). These data indicate that δ TCAR-3 is δ chain specific.

δ TCAR-3 not only immunoprecipitates $\gamma\delta$ TCR heterodimer from the PEER and Molt-13 cell lines, it also binds to the surface of these cell lines and to the IDP2 clone (Brenner, M., et al., 1987, Nature 325:689-694). It does not bind to the $\alpha\beta$ TCR-bearing HPB-ALL and Jurkat cell lines (Fig. 8). In contrast, WT31 (Tax, W.J.M., et al., 1983, Nature 304:445-447), a framework monoclonal antibody to the $\alpha\beta$ TCR, reacts with $\alpha\beta$ TCR-positive HPB-ALL and Jurkat cell lines, but not with $\gamma\delta$ TCR-positive Molt-13, PEER, and IDP2 cells (Fig. 8). When normal peripheral blood lymphocytes (PBL) were examined, a subpopulation (0.9-2.4%) of CD3⁺ lymphocytes were positive with δ TCAR-3 (Fig. 9). When δ TCAR-3, immobilized on tissue culture plates was used for culture of normal human PBL, it selectively stimulated the proliferation of the $\gamma\delta$ TCR-positive subpopulation. After 45 days in culture, the $\gamma\delta$ TCR subpopulation represented 96% of the total cell count.

Antibodies to the $\alpha\beta$ T cell antigen receptor stimulate a rise in the cytoplasmic free calcium ion concentration $[\text{Ca}^{2+}]_i$ (Weiss, A., et al., 1986, Ann. Rev. Immunol. 4:593). Incubation of Molt-13 cells with δ TCAR-3 elicited a rapid increase in $[\text{Ca}^{2+}]_i$ similar to the response induced by anti-T3 antibodies (Fig. 10). Moreover, δ TCAR-3 similarly stimulated a Ca^{2+} flux in PEER cells and in the $\gamma\delta$ TCR-positive cell line generated from PBL as described above. We have also observed that incubation of Molt-13, PEER, and IDP2 cells with δ TCAR-3 causes the co-modulation of the CD3 protein complex.

Further characterization of the epitope specificity of mAb δ TCAR-3 (also termed mAb TCS δ 1) is presented in Section 11.2.2, *infra*.

8. THREE FORMS OF THE HUMAN T CELL RECEPTOR $\gamma\delta$: PREFERENTIAL USE OF ONE FORM IN SELECTED HEALTHY INDIVIDUALS

In the examples herein, the structure of a new form of the human T cell receptor $\gamma\delta$ ($\gamma\delta$ TCR), consisting of a 40 kD TCR γ glycoprotein noncovalently associated with a TCR δ chain, is presented. The newly identified γ TCR glycoprotein, termed Form 2bc, differs in size by more than 15 kD (40 kD versus 55 kD) compared to the previously described nondisulfide-linked TCR γ form (Form 2abc). This difference is accounted for by a 5 kD smaller polypeptide backbone size (35 kD versus 40 kD) and by a reduction in the amount of carbohydrates (5 kD versus 15 kD). Nucleotide sequence analysis of cDNA clones corresponding to Form 2bc revealed that Form 2bc cDNA clones lacked one copy of the constant region (C γ 2) second exon that is present in the cDNA of the other nondisulfide-linked TCR γ subunit (Form 2abc). This CII exon copy encodes part of a connector region between the membrane spanning region and the extracellular constant domain. Since the number and localization of the potential N-linked carbohydrate attachment sites is the same in both nondisulfide-linked forms, we conclude that the connector region influences the amount of attached carbohydrates, probably by affecting the conformation of the protein. In contrast, the δ TCR subunits of these $\gamma\delta$ TCR forms show little variability in peptide backbone sizes or peptide mapping analyses.

We also examined the usage of the three forms of the $\gamma\delta$ TCR complex in peripheral blood. Nearly exclusive use of the disulfide-linked form, Form 1, was observed in certain healthy subjects. In some individuals, Form 1 was expressed together with Form 2bc. Form 2abc was not identified in the subjects tested.

8.1. EXPERIMENTAL PROCEDURES

8.1.1. ANTIBODIES

5 Monoclonal antibodies used were anti-Leu4 (anti-CD3) (Ledbetter et al., 1981, J. Exp. Med. 153:310-323), β F1 (anti- β TCR) (Brenner et al., 1987, J. Immunol. 138:1502-1509), anti-TCR δ 1 (anti- δ TCR) (described in Section 6, *supra*; reactive with the δ TCR chain constant region), P3 (control) (secreted by P3X63.Ag8; Koehler and Milstein, 1975, Nature 256: 495-497), 187.1 (rat anti-mouse κ light chain) (Yelton et al., 1981, Hybridoma 1:5-11), and WT31 (stains $\alpha\beta$ TCR lymphocytes brightly) (Spits et al., 1985, J. Immunol. 135:1922-1928). Anti-Cyb peptide serum (anti- γ TCR) was generated against a 22 amino acid synthetic peptide (Gln-Leu-Asp-Ala-Asp-Val-Ser-Pro-Lys-Pro-Thr-Ile-Phe-Leu-Pro-Ser-Ile-Ala-Glu-Thr-Lys-Cys) (PCT International Publication No. WO 88/00209, published January 14, 1988).

8.1.2 CELL LINES

15 PEER (Weiss et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:6998-7002) and Molt-13 (isolated by J. Minowada, Loh et al., 1987, Nature 330:569-572) are T leukemic cell lines. Umbilical cord blood derived clone WM-14 (Alarcon et al., 1987, Proc. Natl. Acad. Sci. U.S.A. 84:3861-3865) and peripheral blood derived cell line IDP2 (Brenner et al., 1986, Nature 322:145-149; PCT International Publication No. WO 88/00209, published January 14, 1988) and thymus-derived Clone II (Bank et al., 1986, Nature 322:179-181) were cultured as described earlier. Peripheral blood derived cell line 2 (PBL-L2) was isolated by sorting peripheral blood isolated lymphocytes that did not stain with mAb WT31. The isolated cells were then expanded *in vitro* in RPMI 1640 medium supplemented with 10% (v/v) conditioned medium containing IL-2 and 10% (v/v) human serum, and stimulated every 3 weeks with irradiated autologous feeder cells.

8.1.3 IODINATION AND IMMUNOPRECIPITATION

25 2 x 10⁷ cells were isolated by Ficoll-diatrizoate (Organon Teknika Corp.) centrifugation and iodinated on ice in 0.5 ml of phosphate-buffered saline, pH 7.4 (PBS) containing 1 mM MgCl₂, 5 mM glucose by adding 100 μ g of lactoperoxidase (80-100 U/mg, Sigma) and 1 mCi of Na¹²⁵I (New England Nuclear). Ten μ l of a 0.03% hydrogen peroxide solution was added at 5 minute intervals over a reaction period of 30 minutes. Cells were solubilized overnight in detergent supplemented TBS (50 mM Tris-Base pH 7.6, 140 mM NaCl) containing 1 mM phenylmethylsulfonyl fluoride (PMSF, Sigma) and 8 mM iodoacetamide (IAA, Sigma). As indicated, different detergents used in this study were 0.3% (w/v) 3-[(3-cholamidopropyl) dimethylammonio] 1-propane-sulfonate (CHAPS, Sigma). 1% (w/v) digitonin (Aldrich) and Triton X-100 (TX-100, Sigma). After 20 minutes of centrifugation at 10,000 x g to remove insoluble material, detergent lysates were precleared by a 30 minute incubation with 4 μ l of normal rabbit serum (NRS) and 400 μ l of 187.1 hybridoma culture medium, followed by addition of 200 μ l of a 10% (w/v) cell suspension of fixed *Staphylococcus aureus* Cowan I (Pansorbin, Calbiochem). After a one-hour incubation, Pansorbin was removed by centrifugation. Specific precipitations were carried out by adding 0.25 μ l β F1 ascites, 1 μ l 1 mg/ml anti-Leu4 or 0.25 μ l P3 ascites, together with 150 μ l of 187.1 culture supernatant to each sample, followed by a one-hour incubation. 100 μ l of 10% (v/v) Protein A-Sepharose (Pharmacia) was added and the mixture was rocked for 1 hour at 4°C. Immunoprecipitates were washed five times with 0.1% (v/v) Triton X-100 containing TBS and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970, Nature 227:680-685).

45 For immunoprecipitations with the anti-Cyb peptide serum, iodinated cells were solubilized in 1% (w/v) sodium dodecyl sulfate (SDS) containing TBS and then boiled for 3 minutes. After cooling, 5 volumes of 2% (v/v) Triton X-100 in TBS containing PMSF and IAA was added, together with 200 μ l of a mixture of 1 mg/ml deoxyribonuclease (DNase) and 0.5 mg/ml RNase in 50 mM MgCl₂. Preclearing and immunoprecipitations were performed as described above, omitting the addition of 187.1 mAb. Immunoprecipitates were washed in TBS containing 0.5% (v/v) TritonX-100, 0.5% (w/v) deoxycholate (DOC), 0.05% (w/v) SDS. Triton and Sepharose are trade marks.

8.1.4. BIOSYNTHETIC LABELLING

55 4 x 10⁷ exponentially growing cells were resuspended in 4 ml of methionine and cysteine-free RPMI 1640 (Select-Amine kit, Gibco) supplemented with 10% dialyzed fetal calf serum (FCS) and 20 mM Hepes. After a 30 minute starvation period at 37°C, 1 mCi of ³⁵S-methionine and 1 mCi of ³⁵S-cysteine were added, allowing a 15 minute labelling period. Cells were harvested and solubilized in 2% (v/v) Triton X-100, TBS. Preclearing

and immunoprecipitations were performed as described above. The immunoprecipitates were washed four times in 0.5% (v/v) Triton X-100, 0.5% (w/v) deoxycholic acid, 0.05% (w/v) SDS, TBS followed by three washes in 0.5% (v/v) Triton X-100, 0.5 M NaCl, 5 mM EDTA, 50 mM Tris, pH 7.6. The samples were analyzed by SDS-PAGE and visualized by standard fluorography procedures (Bonner and Laskey, 1974, Eur. J. Biochem. 46:83-88).

8.1.5. GEL PURIFICATION OF δ TCR PROTEINS

Surface iodinated cells were solubilized in 0.3% (w/v) CHAPS-TBS and immunoprecipitated using 50 μ l of anti-Leu4-coupled Sepharose beads. The immunoprecipitated species were resolved by SDS-PAGE under nonreducing conditions and the wet gel was exposed for 24 hours at 4°C on XAR-5 film (Kodak) to visualize radiolabelled δ TCR proteins. The gel regions corresponding to δ TCR were excised, incubated in 5% (v/v) 2-mercaptoethanol containing sample buffer and resolved a second time by SDS-PAGE. Because of the characteristic SDS-PAGE mobility shift upon reduction, δ TCR protein could be separated and then purified from contaminants. TCR proteins were eluted from gel slices by overnight incubation in 0.05% (w/v) SDS, 50 mM ammonium bicarbonate buffer at 37°C and lyophilized.

8.1.6. ENDOGLYCOSIDASE DIGESTION

For endoglycosidase H (Endo H) digestions, immunoprecipitated material or gel purified protein was boiled for 3 minutes in a 40 μ l of 1% (w/v) SDS solution containing 0.14 M 2-mercaptoethanol. After cooling, the mixture was diluted with 360 μ l of 0.15 M acetate buffer, pH 5.5 containing 1 mM PMSF. Five μ l Endo H (1 U/ml Endo- β -N-acetylglucosaminidase H, Genzyme) was incubated with half of the above solution for 14 hours at 37°C, while the other half was mock treated.

For N-glycanase (N-GLY) digestion, gel purified material was boiled for 3 minutes in 35 μ l of 0.5% (w/v) SDS, 0.10 M 2-mercaptoethanol. Then, 100 μ l of 0.2 M sodium phosphate (pH 8.6), 1.25% (v/v) Triton X-100 was added. Half of the mixture was incubated with 1 μ l of N-Glycanase (250 U/ml, peptide-N-[N-acetyl- β -glucosaminyl]asparagine amidase; Genzyme) and incubated for 16 hours at 37°C, while the other half was mock treated.

After digestion, 10 μ g bovine serum albumin was added as carrier and samples were recovered by trichloroacetic acid precipitation. Protein pellets were taken up in sample buffer containing 5% (v/v) 2-mercaptoethanol.

8.1.7. PRODUCTION OF MONOCLONAL ANTIBODY anti-Cym1

Part of the Cy C1 and CII exons of HPB-MLT pTy-1 was isolated using the *Bam*HI and *Pst*I sites at nucleotide positions 571 and 848 (Dialynas et al., 1986, Proc. Natl. Acad. Sci. USA 83:2619-2623) and was cloned into expression vector pRIT2T (Pharmacia). The resulting Protein A fusion protein was expressed in *E. coli* N4830. Bacteria were lysed with lysozyme and the fusion protein was isolated by purification over a IgG Sepharose column. Mice were injected intraperitoneally with 100 μ g of fusion protein in Freund's adjuvant at days 0, 7 and 28. Twenty-eight days later 100 μ g of fusion protein in PBS was injected intravenously. After three days, splenocytes were isolated and fused with the hybridoma P3X63Ag8.653 as described (Brenner et al., 1987, J. Immunol. 138:1502-1509). Hybridomas were screened by enzyme-linked immunoabsorbent assay (ELISA). Ninety six-well flat bottom plates (LINBRO, Flow Laboratories) were incubated overnight with 0.4 μ g of fusion protein or nonfused protein in PBS. Nonspecific binding sites were blocked at 23°C with 0.25 mg/ml normal rabbit IgG (Sigma) in PBS containing 50% (v/v) FCS. 50 μ l of hybridoma supernatant was added for 1 hour at 4°C, followed by a similar incubation in 50 μ l of a 5 μ g/ml solution of peroxidase-conjugated anti-mouse IgG (Cappel). All described incubations were interspersed with washing steps, using 10% (v/v) FCS, 0.1% (w/v) BSA, PBS. The ELISA was developed with 0.08% (w/v) O-phenylenediamine (Sigma) in 0.012% (w/v) hydrogen peroxide containing phosphate-citrate buffer, pH 5.0.

Although anti-Cym1 (IgG₁) does not recognize the native $\gamma\delta$ TCR/CD3 complex in cytofluorographic analysis nor the $\gamma\delta$ TCR heterodimer from Triton X-100 solubilized cells in immunoprecipitation, it does recognize biosynthetically labelled γ TCR precursor and mature γ TCR proteins after separation of CD3/ $\gamma\delta$ TCR proteins into individual chains. In this way, anti-Cym1 was shown to recognize the γ TCR protein after separating CD-3/ $\gamma\delta$ TCR complexes into individual chains by boiling anti-CD3 immunoprecipitates in 1% (w/v) SDS in TBS (Fig. 12, lane 3).

8.1.8. ISOLATION AND SEQUENCING OF A MOLT-13 γ TCR cDNA CLONE

Poly (A)⁺ RNA was prepared from MOLT-13 cells by urea/lithium chloride precipitation followed by oligo (dT) cellulose affinity chromatography. A λ gt 10 cDNA library was prepared from poly(A)⁺ RNA by the method of Huynh et al., 1985 (DNA Cloning, Glover, D.M. ed. IRL Press, Oxford, 1:49-78) using Mung Bean Nuclease for the hairpin loop cleavage (McCutcham et al., 1984, Science 225:626-628). The cDNA library was amplified on the *E. coli* strain C600 Hf1 and screened by plaque filter hybridization with ³²P-labelled PT γ l (Dialynas et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:2619-2623). Positive clones were analyzed for size and restriction enzyme map, and cDNA clone M13k was selected for sequencing. The cDNA of M13k was excised from λ gt 10 phage with the endonuclease *Eco*RI and further digested with appropriate restriction enzymes. The fragments were subcloned into M13 vectors and sequenced by the dideoxy chain termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467) using the modified T7 polymerase (Sequenase, United States Biochemical Corp.).

Clone M13k corresponds to a full length, in frame, γ TCR transcript, including 36 nucleotides of 5' untranslated region and 72 nucleotides of 3' noncoding region (Fig. 14). The nucleotide sequence of the V region is identical to the genomic V γ 1.3 sequence (nomenclature Lefranc et al., 1986a, Cell 45:237-246; Strauss et al., 1987, Science 237:1217-1219), except for a C to T (Ile to Val) change of nucleotide 53 in the putative signal sequence. The J region is identical to the J γ 2.3 sequence (nomenclature based on Lefranc et al, 1986b, Nature 319:420-422; Quertermous et al, 1987, J. Immunol. 138:2687-2690). Interestingly, 8 nucleotides occur at the V-J junction which do not appear to be encoded by the genomic V or J sequences and presumably represents an N-region. The C region sequences match the corresponding genomic sequence (Lefranc et al., 1986c, Proc. Natl. Acad. Sci. U.S.A. 83:9596-9600), with the exception of nucleotide 559 (G to C; Val to Ile) and nucleotide 908 (T to C; Met to Thr).

8.2. RESULTS

8.2.1. NOVEL $\gamma\delta$ TCR PROTEIN COMPLEX

Preliminary studies of peripheral blood $\gamma\delta$ TCR lymphocytes revealed the presence of a CD3-associated complex that was different from the known human $\gamma\delta$ TCR forms. In an attempt to delineate this form, we produced and characterized a number of cell lines derived from normal human donors. Peripheral blood lymphocytes were stained with monoclonal antibody (mAb) WR31, which brightly stains resisting $\alpha\beta$ TCR lymphocytes. Cells that did not stain were isolated by cell sorting and then expanded *in vitro* in IL-2 containing medium. Peripheral blood lymphocyte line 2 (PBL-L2) obtained in this way, proved to be homogeneously CD3⁺CD4⁻CD8⁻, a cell surface phenotype characteristic of $\gamma\delta$ TCR lymphocytes.

To visualize $\gamma\delta$ TCR complexes on PBL-L2 cells, immunoprecipitations with an anti-CD3 mAb were carried out from cell surface ¹²⁵I-labelled cells solubilized in CHAPS or digitonin. In these detergents, the physical association between the CD3 complex and $\gamma\delta$ TCR subunits is preserved. SDS-PAGE of anti-CD3 immunoprecipitates from PBL-L2 cells resolved 40 kD and 44 kD proteins (referred to as 40 kD) that were identified as γ TCR subunits by anti-C γ b serum, an antiserum directed against a γ TCR constant region peptide (Fig. 11A; see methods section).

These γ TCR proteins on PBL-L2 are noncovalently associated with a δ TCR subunit, which is visible as a weakly iodinated protein in the anti-CD3 immunoprecipitate analyzed under nonreducing conditions (Fig. 11A, lane 6, closed arrow). This weakly iodinated protein represents the δ TCR subunit on PBL-L2 cells, since it is not recognized by anti-C γ b serum (Fig. 11A, lane 8). In addition, it displays the same SDS-mobility shift comparing analysis under nonreducing and reducing conditions as was noted for the δ TCR proteins on IDP2 and PEER cells (see *infra*; see also PCT International Publication No. WO 88/00209, published January 14, 1988). The δ TCR protein could not be visualized after reduction (Fig. 11A, lane 3), because it migrated with a mobility of 40 kD (see *infra*) and then was obscured by the similar sized γ TCR protein (open arrow).

This $\gamma\delta$ TCR form is not only present on normal peripheral blood T lymphocytes, but is also observed on thymus-derived Clone II cells (Fig. 11D) and on the T-leukemic cell line MOLT-13 (Fig. 11E). These three cell lines possess γ TCR species that display differential glycosylation resulting in a γ TCR protein doublet observed on PBL-L2 (40 kD and 44 kD; Fig. 11A, lane 8) and Clone II cells (40 kD and 44 kD, 11D, lane 8) or a diffusely labelled γ TCR protein band observed on MOLT-13 cells (40 to 46 kD; Fig. 11E, lane 6). Two-dimensional gel analysis [nonequilibrium pH gradient electrophoresis (NEPHGE) followed by SDS-PAGE] of the MOLT-13 γ TCR protein band resolved two parallel γ TCR species (40 kD and 44 kD), of which the 44 kD γ TCR species contained an additional high mannose (or hybrid) N-linked glycan compared to the 40 kD γ TCR species. Thus, the γ TCR subunits of this receptor complex isolated from three different cell sources (peripheral blood, thymus,

and leukemia), revealed cell surface species of 40 kD that are noncovalently associated with γ TCR partner chains.

For comparison to the $\gamma\delta$ TCR form on PBL-L2, Clone II and MOLT-13 cells, we examined the previously known forms on the IDP2 and WM-14 cell lines. The IDP2 cell line (see PCT International Publication No. WO 88/00209, published January 14, 1988; Brenner et al., 1986, Nature 322:145-149) contains a larger, 55-60 kD γ TCR protein (referred to as 55 kD), which is recognized by anti-C γ b serum (Fig. 11B). When the anti-CD3 immunoprecipitate is examined under nonreducing conditions, it is evident that the IDP2 γ TCR protein is associated noncovalently with its γ TCR partner chain (Fig. 11B, lane 4, solid arrow). Upon reduction, the δ TCR protein displays a decrease in SDS-PAGE mobility to a relative molecular mass of 40 kD (compare Fig. 11B, lane 4, closed arrow, with Fig. 11B, lane 2, open arrow).

In contrast to the noncovalently associated $\gamma\delta$ TCR forms, the peripheral blood-derived T cell clone, WM-14, bears a disulfide-linked TCR dimer of 70 kD (Fig. 11C, lane 7), that was recognized by anti-C γ serum (Alarcon et al., 1987, Proc. Natl. Acad. Sci. U.S.A. 84:3861-3865). This dimer is also recognized by anti-TCR δ 1, a mAb directed against the δ TCR subunit (Fig. 11C, lane 5), and therefore represents a $\gamma\delta$ TCR heterodimer. Analysis under reducing conditions reveals three γ TCR proteins of 36 kD, 40 kD and 43 kD (referred to as 40 kD).

Thus, the CD3-associated complex on PBL-L2, Clone II and MOLT-13 cells constitutes a novel $\gamma\delta$ TCR heterodimer compared to the previously known forms, since its TCR γ subunit is 40 kD (similar in size to the disulfide-linked C γ 1 encoded γ TCR protein on WM-14 cells), yet it is not disulfide-linked to its partner chain (similar to the 55 kD, C γ 2 encoded γ TCR protein on IDP2 cells). To understand the molecular basis of this complex, more detailed structural analysis of its γ TCR and δ TCR subunits was carried out as described *infra*, using the MOLT-13 cell line as an example.

8.2.2. CORE POLYPEPTIDE SIZE OF MOLT-13 γ TCR SUBUNIT

To determine the size of the γ TCR core polypeptide of MOLT-13 cells (40 kD γ TCR glycoprotein), and compare it with that of PEER cells (55 kD γ TCR glycoprotein), both cell lines were biosynthetically labelled for 15 minutes in the presence of 35 S-methionine and 35 S-cysteine, solubilized in Triton X-100 and then immunoprecipitated with anti-C γ m1, a monoclonal antibody that specifically recognizes the γ TCR chain (Fig. 13A, see methods section). Immunoprecipitated material was subsequently digested with endoglycosidase H (Endo H) to remove the immature N-linked glycans. The MOLT-13 γ TCR polypeptide backbone has a relative molecular mass of 35 kD (Fig. 13A, lane 8), which is 5 kD smaller than the PEER γ TCR core polypeptide (40 kD; Fig. 13A, lane 4) or the IDP2 γ TCR core polypeptide (40 kD; See PCT International Publication No. WO 88/00209, published January 14, 1988). It now can be concluded that MOLT-13 cells express a γ TCR core polypeptide that is distinct from the IDP2 and PEER γ TCR core polypeptides based on its being 5 kD smaller in size. In addition, only 5-11 kD of size on the mature MOLT-13 γ TCR cell surface glycoprotein are accounted for by post-translational processes (40-46 kD surface size minus 35 kD core size), where 15-20 kD of relative molecular mass can be accounted for by post-translational processes on the PEER and IDP2 γ TCR glycoproteins (55-60 kD surface size minus 40 kD core size). Assuming that all post-translational processes are N-linked glycans and that each glycan chain accounts for approximately 3 kD of relative molecular mass, we predict that 2 to 3 N-linked glycans are attached to the MOLT-13 γ TCR protein, while 5 N-linked glycans are added to the polypeptides on PEER and IDP2 cells. Experiments using N-glycanase to remove N-linked carbohydrates from cell surface γ TCR proteins showed that the majority of the post-translational processes that are added to the core polypeptide are indeed N-linked glycans.

8.2.3. PRIMARY SEQUENCE OF MOLT-13 γ TCR

To understand the structure of the constant region gene segment encoding the MOLT-13 γ TCR subunit, the sequence of a cDNA clone representing the MOLT-13 γ TCR transcript was determined. A λ gt10 library from MOLT-13 derived poly-A⁺ RNA was constructed and probed with a human γ TCR cDNA clone, pT γ -1 (Dialynas et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:2619-23). Based on size and limited restriction enzyme mapping, one clone, M13k, was selected and its nucleotide sequence determined (Fig. 14). Clone M13k represents a full length, in-frame γ TCR transcript, using a V γ 1.3 gene segment joined to a J γ 2.3 gene segment (Lefranc et al., 1986, Cell 45:237-246; Lefranc et al., 1986, Nature 319:420-422; nomenclature based on Strauss et al., 1987, Science 237:1217-1219; Quertermous et al., 1987, J. Immunol. 138:2687-2690). The constant region sequence was found to be nearly identical to a recently reported non-functional γ TCR (Pellici et al., 1987, Science 287:1051-1055) and to the C γ 2 genomic sequence containing two CII exon copies b and c (Lefranc et al., 1986, Proc. Natl. Acad. Sci. U.S.A. 83:9596-9600) (see methods section for detailed account). This represents the first in-frame transcript encoding a γ TCR protein expressed on the cell surface that utilizes a C γ 2

gene segment with two CII exon copies.

The deduced amino acid sequence of this cDNA clone predicts a polypeptide backbone size of 34.8 kD which is in good agreement with biochemical data described above. Surprisingly, six potential N-linked carbohydrate attachment sites are encoded by this transcript. Since the biochemical data suggest that only 2 to 3 N-linked glycans are attached to the polypeptide chain, it indicates that not all potential sites are used.

To reflect γ gene segment usage, we have denoted the disulfide-linked $\gamma\delta$ TCR form expressed by PBL-C1 and WM-14 as "Form 1", since such disulfide-linked γ TCR chains utilize the γ 1 gene segment (Kragel et al., 1987, Science 237:64-67). The large (55 kD), nondisulfide-linked γ TCR subunit of the $\gamma\delta$ TCR form expressed on IDP2 and PEER cells is encoded by γ 2 gene segments containing three CII exon copies, namely copy a, copy b and copy c (Kragel et al., 1987, Science 237:64-67; Littman et al., 1987, Nature 326:85-88) and therefore this $\gamma\delta$ TCR form is called herein "Form 2abc". In concordance, the form characterized on MOLT-13 cells is referred to as "Form 2bc".

8.2.4. PREFERENTIAL γ GENE SEGMENT USAGE

To determine the presence of these three $\gamma\delta$ TCR forms in freshly isolated peripheral blood we analyzed the mononuclear cells from ten healthy subjects, using biochemical analysis with mAb anti-TCR δ 1 (described in Section 6, *supra*; reactive with the δ TCR constant region). This antibody reacts with the great majority, if not all $\gamma\delta$ TCR lymphocytes. Representative results from this panel are shown in Figure 15. In subject 1, anti-TCR δ 1 immunoprecipitates (analyzed under nonreducing conditions) demonstrate the presence of both disulfide-linked $\gamma\delta$ TCR complexes as a 70 kD protein band (Form 1) and nondisulfide-linked $\gamma\delta$ TCR complexes as a broad 40 kD protein band (Form 2bc) (Fig. 15, lane 2). This indicates that the γ 1 and γ 2 constant regions are both used by the expressed $\gamma\delta$ TCR of this individual. However, the amount of Form 2bc varied among individuals. Note the smaller fraction of Form 2bc in subject 2 compared to subject 1 by comparing the intensity of the 40 kD protein bands in both individuals (compare lane 2 of subject 2 with lane 2 of subject 1). Even more strikingly, only disulfide-linked $\gamma\delta$ TCR complexes could be detected on the mononuclear cells of three of the ten individuals examined, even after long exposure of the autoradiographs (see subject 3). None of the analyzed individuals revealed the 55 kD, nondisulfide-linked $\gamma\delta$ TCR complex (Form 2abc) in peripheral blood.

8.2.5. CHARACTERIZATION OF THE δ TCR SUBUNIT

In contrast to the striking structural differences in size and glycosylation of the γ TCR proteins, δ TCR subunits from different cell sources proved to be markedly similar. The relative molecular mass of the δ TCR glycoprotein on MOLT-13 cells was directly determined to be 40 kD using the anti- δ TCR mAb (Fig. 12, lane 4), confirming that it is similar in size to the δ TCR glycoprotein on IDP2 cells (Fig. 11B, lane 2, open arrow).

To also compare δ TCR polypeptide backbone sizes, cell surface 125 I-labelled δ TCR protein from MOLT-13 cells was digested with N-glycanase to remove asparagine-linked glycans (of the high mannose, hybrid, and complex-type; Tarentino et al., 1985, Biochem. 24:4665-4671; Hirani et al., 1987, Anal. Biochem. 162:485-492). The δ TCR core polypeptides of MOLT-13 cells has a relative molecular mass of 35 kD (Fig. 13B, lane 4), which is similar to that of the δ TCR backbone of IDP2 cells (35 kD) (Band et al., 1987, Science 238:682-684).

In addition, digestion of cell surface 125 I-labelled MOLT-13 δ TCR protein with endoglycosidase H (Endo H, removing only high mannose and certain hybrid N-glycans; Tarentino et al., 1974, J. Biol. Chem. 249:811-817; Trimble and Maley, 1984, Anal. Biochem. 141:514-522) caused a decrease in relative molecular mass of 2.5 kD, (Fig. 13B, lane 2) consistent with the presence of one carbohydrate moiety, leaving a relative mass of 2.5 kD of Endo H resistant carbohydrates attached to the polypeptide. Since there are two potential N-glycan attachment sites present in the δ TCR constant domain (Hata, S., et al., 1987, Science 238:678-682; Loh et al., 1987, Nature 330:569-572), these data show that both are used, but that their N-glycans are processed differently, namely one as a high mannose N-glycan (Endo H-sensitive) and the other as a complex N-glycan (Endo H-resistant, but N-glycanase sensitive). In contrast to the different amounts of attached N-linked carbohydrate on γ TCR polypeptide chains, the δ TCR subunits expressed on PEER, IDP2 and MOLT-13 cells all revealed the same peptide core sizes and the presence of two N-linked glycans (Fig. 13B and data not shown).

8.3. DISCUSSION

In this example, three protein forms of the human γ TCR glycoprotein are compared, namely the disulfide-linked 40 kD γ TCR protein (Form 1), the nondisulfide-linked 55 kD γ TCR protein (Form 2abc) and the nondisulfide-linked 40 kD γ TCR protein (Form 2bc). All three forms are shown to be associated with a δ TCR subunit.

Complementary DNA sequences representing the first two γ TCR forms have been reported previously (Kragel et al., 1987, Science 237:64-67; Littman et al., 1987, Nature 326:85-88. The constant region of γ TCR polypeptide Form 1 (on PBL-C1) is encoded by the $C\gamma 1$ gene segment containing a single CII exon, while γ TCR polypeptide Form 2abc (on IDP2 and PEER cells) utilizes the $C\gamma 2$ gene segment containing CII exon copy a, copy b and copy c. The cDNA sequence corresponding to a γ TCR chain of Form 2bc was shown to contain a $C\gamma 2$ gene segment utilizing only two CII exon copies, namely copy b and copy c. Similarly, it seems likely that the gene structure of the γ TCR connector region of Clone II and PBL-L2 (nondisulfide-linked, 40 kD γ TCR protein) will also be like the MOLT-13 structure determined here, namely of Form 2bc. Since the δ TCR constant region used is the same for all these forms (Hata, S., et al., 1987, Science 238:678-682; Loh, E.Y., et al., 1987, Nature 330:569-572), a complete comparison of the structures of the three $\gamma\delta$ TCR forms in man now can be made (Fig. 16).

Two $C\gamma 2$ polymorphic genomic forms exist in man (Lefranc et al., Nature 319:420-422; Pellici et al., 1987, Science 237:1051-1055). The two transcript forms (Form 2abc and Form 2bc) are probably the product of these different allelic types. To date, no allelic form of γ TCR polypeptides have been found in mice. We conclude that the dramatic difference in γ TCR cell surface protein size between Form 2abc (55 kD) and Form 2bc (40 kD) is largely determined by the amount of attached N-link carbohydrates, most likely reflecting the number of N-linked glycans. Backbone sizes of IDP2 γ TCR (Form 2abc) and MOLT-13 γ TCR (Form 2bc) proteins have been measured to be 40 kD and 35 kD respectively, on the basis of SDS-PAGE, which correlates well with their predicted molecular masses of 36.6 kD and 34.8 kD respectively, calculated on the basis of cDNA sequences. It is clear that this small difference in backbone size (5 kD in SDS-PAGE), accounted for mainly by one CII exon encoded peptide of 16 amino acids, contributed to, but could not solely explain the observed difference in molecular mass between the 55 kD and 40 kD nondisulfide-linked γ TCR surface forms. Form 2abc γ TCR polypeptides possess 5 potential N-linked glycan attachment sites that are probably all used, in contrast to the MOLT-13 γ TCR polypeptide which bears one additional potential attachment site, while carrying only 2 to 3 N-linked glycans. The reason for this limited use of potential attachment sites is unknown, but may result from the influence of the CII exon encoded peptides on the conformation of the γ TCR protein. The CII exon encoded peptides and their neighboring amino acids make up a connector region between the plasma membrane and the immunoglobulin-like constant domain. This region contains most of the N-linked glycan attachment sites (Fig. 17). We conclude that the CII exon copies appear to determine the protein form not only by determining polypeptide backbone size, and by creating the ability to disulfide-link chains, but also by influencing the amount of attached carbohydrates.

δ TCR complementary DNAs of IDP2 (Hata et al., 1987, Science 238:678-682), PEER (Loh et al., 1987, Nature 330:569-572) and MOLT-13 cells have been sequenced and were found to be identical, except for the diversity/N-region interspersing the variable and constant region gene segments. The δ TCR protein on WM-14 cells has a relative molecular mass of 43 kD, which is similar to the δ TCR protein described previously (Borst et al., 1987, Nature 325:683-688; Lanier et al., 1987, J. Exp. Med. 165:1076-1094) but is 3 kD larger than the other δ TCR chains. These 43 kD δ TCR proteins might indicate the presence of an additional N-linked glycosylation site in a different δ variable domain.

Structural differences comparable to those described for γ TCR constant region segments have not been observed for α TCR and β TCR genes (Yoshikai et al., 1985, Nature 316:837-840; Toyonaga et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82:8624-8628; Royer et al., 1984, J. Exp. Med. 160:947-952; Kronenberg et al., 1985, Nature 313:647-653). There is possible structural similarity in the number of human CII exon repeats with the length in murine $C\gamma$ regions, of which the $C\gamma 1$, $C\gamma 2$ and $C\gamma 4$ constant regions encode for 15, 10 and 33 amino acid connector region respectively (Garman et al., 1986, Cell 45:733-742; Iwamoto et al., 1986, J. Exp. Med. 163:1203-1212). The connector regions in mouse, however, reflect a difference in the size of the relevant exon, not the multiple use of exons as is seen in Form 2abc γ TCR and Form 2bc γ TCR in humans. Also, the murine γ TCR only exist in disulfide linked forms in contrast to the two nondisulfide linked human forms.

Importantly, the human $\gamma\delta$ TCR forms do not appear to be used equally. In some individuals (selected for high percentages of $\gamma\delta$ TCR lymphocytes), a single form (Form 1) predominates, suggesting that either positive selection occurs for this form or that there is selection against other $\gamma\delta$ TCR forms.

9. THREE T CELL RECEPTOR $\gamma\delta$ ISOTYPIC FORMS RECONSTITUTED BY PAIRING OF DISTINCT TRANSFECTED γ TCR CHAINS WITH A SINGLE δ TCR SUBUNIT

As described in the example herein, the role of the γ TCR polypeptide in the formation of the $\gamma\delta$ heterodimer was explored. We examined by transfection the $\gamma\delta$ TCR complexes formed by the association of γ TCR chains corresponding to the three $\gamma\delta$ TCR forms (Forms 1, 2abc, and 2bc) with a single resident δ TCR chain. γ TCR DNA encoding either Form 1 or Form 2abc of the γ TCR polypeptide was transfected into the MOLT-13 cell line,

which constitutively expresses a $\gamma\delta$ heterodimer comprised of form 2bc γ TCR polypeptide noncovalently associated with δ TCR polypeptide. Transfected cells were capable of expressing, together with the $\gamma\delta$ TCR characteristic of the MOLT-13 cell line, $\gamma\delta$ heterodimers comprised of either Form 2abc γ TCR noncovalently associated with δ TCR or Form 1 γ TCR covalently linked to δ TCR. Furthermore, the glycosylation of the transfected γ TCR gene products was identical to the glycosylation of these genes in their native cell lines. Thus, the degree of glycosylation and the ability to form disulfide linkages are properties determined by the γ TCR gene. γ TCR constant region CII exon usage determines not only the presence or absence of disulfide linkage between TCR γ and δ polypeptides, but also the amount of carbohydrate attached to the γ TCR chain, which is largely responsible for the differences in size of the cell surface γ TCR proteins.

9.1. MATERIALS AND METHODS

9.1.1. CELL LINES

MOLT-13, a TCR $\gamma\delta^+$ T leukemia cell line (Hata, S., et al., 1987, Science 238:678-682; Loh, E.Y., et al., 1987, Nature 330:569-572), and peripheral blood derived TCR $\gamma\delta^+$ cell lines PBL C1 (Brenner, M.B., et al., 1987, Nature 325:689-694) and IDP2 (Brenner, M.B., et al., 1986, Nature 322:145-149) were cultured as previously described.

9.1.2. ANTIBODIES

The monoclonal antibodies (mAb) used were: Anti-leu-4 (anti-human CD3; IgG1) (Ledbetter, J.A. et al., 1981, J. Exp. Med. 153:310-323), anti-TCR δ 1 (anti-human TCR δ chain constant region; IgG1) (See Section 6; Band, H., et al., 1987, Science 238:682-684), anti-Ti- γ A (anti-V γ 2; IgG2a) (Jitsukawa, S., et al., 1987, J. Exp. Med. 166:1192-1197), anti-Cym1 (anti-human γ TCR constant region; see Section 8.1.7), P3 (IgG1 secreted by the P3X63Ag8 myeloma) (Koehler, G., and Milstein, C., 1975, Nature 256:495-497), and 187.1 (rat anti-mouse κ light chain-specific) (Yelton, D.E., et al., 1981, Hybridoma 1:5-11).

9.1.3. ISOLATION AND SEQUENCING OF MOLT-13 δ TCR cDNA CLONES

A complementary DNA (cDNA) library prepared from MOLT-13 poly A⁺ RNA in the vector λ gt10 (Huynh, et al., 1985, in DNA Cloning, ed. Glover, D.M. (IRL Press, Oxford), Volume 1, pp. 49-78) was screened by hybridization with ³²P-labeled human δ TCR cDNA clone IDP2 O-240/38 (Hata et al., 1987, Science 238:678-682). Clones were selected for detailed analysis on the basis of size and limited restriction enzyme mapping. Nucleotide sequence was determined in M13 vectors by dideoxy chain termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467) using the modified T7 polymerase (Sequenase, United States Biochemical Corp.) (Potter et al., 1984, Proc. Natl. Acad. Sci. U.S.A. 81:7161-7165).

9.1.4. CONSTRUCTION OF EXPRESSION PLASMIDS AND TRANSFECTIONS

γ TCR cDNAs (PBL C1.15 and IDP2.11r) (Kragel, M.S. et al., 1987, Science 237:64-67) were cloned into pFneo mammalian expression vector (Saito, T., et al., 1987, Nature 325:125-130; Ohashi, P., et al., 1985, Nature 316:606-609) downstream from Friend spleen focus forming virus (SFFV) long terminal repeat (LTR), as shown schematically in figure 10B. The plasmid constructs were transfected into MOLT-13 cells by electroporation (Potter, H., et al., 1984, Proc. Natl. Acad. Sci. 81:7161-7165). Transfectants were selected and maintained in medium containing 2 mg/ml of G418 (480 μ g/mg solid by bioassay; GIBCO), and cloned by limiting dilution.

9.1.5. IODINATION AND IMMUNOPRECIPITATION

Cell surface labeling with ¹²⁵I using lactoperoxidase, solubilization in 3-[(3-cholamidopropyl) dimethylammonio] 1-propanesulfonate (CHAPS; Sigma Chemical Co., St. Louis, MO), immunoprecipitation with various antibodies, nonequilibrium pH gradient gel electrophoresis (NEPHGE), and SDS polyacrylamide gel electrophoresis (SDS-PAGE) were performed as described (See Section 8.1.3; Brenner, M.B., et al., 1986, Nature 322:145-149; Brenner, M.B., et al., 1987, Nature 325:689-694). Specific immunoprecipitations were carried out with 1 μ g anti-leu-4, 0.1 μ l anti-TCR δ 1 ascites, or 1 μ l P3 ascites, together with 150 μ l of 187.1 culture supernatant. For anti-T9- γ A, 1 μ l of ascites was used without 187.1.

9.1.6. BIOSYNTHETIC LABELING

Exponentially growing cells were incubated for 30 minutes in methionine- and cysteine-free medium followed by a 15 minute pulse labeling at 37°C with ³⁵S-methionine and ³⁵S-cysteine, and immunoprecipitations were carried out as described in Section 8.1.3, *supra*. Immunoprecipitates were either treated with endoglycosidase-H (endo-H) or were mock-incubated, separated by SDS-PAGE, and visualized by fluorography (Bonner, W.M. and Laskey, R.A., 1974, Eur. J. Biochem. 46:83-88).

9.2. RESULTS

We investigated the products resulting from association of structurally distinct γ TCR gene products with a single δ TCR protein in order to demonstrate the role of the γ TCR gene, and in particular the TCR CII exons, in determining the structural differences between various γ TCR isotypes. For this purpose, MOLT-13, a T leukemia cell line that expresses the 40 kD nondisulfide-linked γ TCR polypeptide (Form 2bc), was used as a recipient for γ TCR chain cDNA clones corresponding to the other two forms of the receptor (Forms 1 and 2abc). Complete sequences of the cDNA clones representing these γ TCR chains are described in Figure 14 (Form 2bc), and in Krangel et al. (1987, Science 237:64-67) (Forms 1 and 2abc) and they are schematically represented in Figure 18A.

9.2.1. A SINGLE FUNCTIONAL δ TCR CHAIN IS PRESENT IN THE MOLT-13 CELL LINE

δ TCR gene rearrangement studies of the MOLT-13 cell line (Hata, S., et al., 1987, Science 238:678-682) suggested that only a single functional δ TCR gene product was expressed in this cell line. However, to demonstrate directly that a single functional transcript for δ TCR is made in MOLT-13 cells, cDNA clones cross-hybridizing with a δ TCR cDNA probe (Hata, S., et al., 1987, Science 238:678-682) were isolated from a MOLT-13 cDNA library prepared in λ gt10 and the sequence of selected cDNA clones was determined. This analysis revealed that MOLT-13 cells express transcripts corresponding to one functionally rearranged and one aberrantly rearranged δ TCR gene. The cDNA clone corresponding to the functionally rearranged δ TCR gene has the same V (V δ 1), J (J δ 1), and C gene segments described earlier for the IDP2 cell line (Hata, S., et al., 1987, Science 238:678-682). The MOLT-13 δ TCR cDNA clone, however, possesses a distinct nucleotide sequence between the V and J gene segments arising from D segment utilization (MOLT-13 probably uses only D δ 2), imprecise joining, and N-region diversity at the V-D and D-J junctions (Hata, S., et al., 1988, Science 240:1541-1544). The MOLT-13 δ TCR cDNA also predicts a cysteine residue in the membrane proximal connector region of the constant gene segment that would be available for disulfide linkage to γ TCR gene products that utilize the Cy1 gene segment. Although the MOLT-13 cell line expresses a nondisulfide-linked γ , δ TCR receptor, the presence of a cysteine residue in the membrane proximal connector region of its δ TCR chain leaves open the possibility that this δ TCR subunit might be capable of participating in either a nondisulfide-linked or a disulfide-linked complex.

9.2.2. γ TCR GENE PRODUCT DETERMINES THE FORM OF THE RECEPTOR

The MOLT-13 cells transfected with γ TCR cDNA constructs were abbreviated as M13.PBL C1 γ (for MOLT-13 cells transfected with PBL C1-derived γ TCR cDNA) and M13.IDP2 γ (for MOLT-13 cells transfected with IDP2-derived γ TCR cDNA). The bulk transfectant cell lines and representative subclones derived from these lines were analyzed by Northern blot analysis with γ TCR (VJC) or V γ 2-specific cDNA probes. In addition to the resident 1.6 kb MOLT-13 γ TCR transcript, a second γ TCR transcript of about 1.8 kb (expected size for a γ TCR transcript initiating in the SFFV LTR of the expression plasmid) was observed in transfectant lines and their clones. The 1.8 kb transcript specifically hybridized with a V γ 2 probe that does not cross-hybridize with the V γ 1.3 present in the resident MOLT-13 γ TCR transcript, and thus the 1.8 kb transcript represents the transcript of the transfected γ TCR cDNAs (which utilize a V γ 2 segment).

To biochemically characterize the γ TCR protein(s) expressed on the surface of the transfectants, a representative clone derived from each line was analyzed by immunoprecipitation of surface iodinated cells with P3 (control), anti-leu-4 (anti-CD3), anti-TCR δ 1 (anti- δ TCR), or anti-TI- γ A mAbS. Anti-TI- γ A Jitsukawa, S., et al., 1987, J. Exp. Med. 166:1192-1197) appears to specifically recognize the γ , δ TCR cells that utilize the V γ 2 gene segment as the variable portion of their γ TCR chains. Untransfected (Fig. 19A) as well as the transfected MOLT-13 cells (Fig. 19B and 19C) express the expected parental γ TCR (40 kD; see open arrow) and δ TCR subunits (see asterisk). Note that anti-V γ 2-specific mAb (anti-TI- γ A) fails to react with the resident MOLT-13 γ TCR chain (Fig. 19, lanes 7 and 8). Anti-CD3 immunoprecipitates of M13.PBL C1 γ transfectant cells revealed

an additional CD3-associated species (68 kD) when examined under nonreducing conditions (Fig. 19B, lane 3, see solid arrow). On both PBL C1 cells (Fig. 19D, lanes 3 and 4) and the M13.PBL C1 γ transfectant cell line (Fig. 19B lanes 3 and 4), the 68 kD complex yielded 40 and 36 kD species upon reduction (in the case of the M13.PBL C1 γ transfectant, these bands are clearly visualized in the anti-V γ 2 immunoprecipitate, see Fig. 19B, lanes 7 and 8, solid arrows). These 40 and 36 kD species represent differentially glycosylated γ TCR polypeptides (Brenner, M.B., et al., 1987, Nature 325:689-694). In these immunoprecipitates, the δ TCR chain (40 kD reduced) comigrates with the 40 kD γ TCR polypeptide and is therefore not visualized (however, see below).

Importantly these experiments show that the resident δ TCR chain of the MOLT-13 cell line, normally part of a nondisulfide-linked complex, associates with the PBL C1 γ TCR protein to form a disulfide-linked γ , δ TCR heterodimer in the transfectant cell line. In contrast, the IDP2-derived γ TCR protein (55 kD) in the M13.IDP2 transfectant cell line formed a nondisulfide-linked complex with the resident MOLT-13 γ TCR chain (Fig. 19C, lanes 3 and 4). Immunoprecipitates carried out with anti-TCR δ 1 mAb (specific for γ TCR peptide) confirmed that the endogenous (Figs. 19A, D and E, lanes 5 and 6) as well as the transfected γ TCR chains (Fig. 19B and C, lanes 5 and 6) from all these cell lines were associated directly with the γ TCR chain. Anti-Ti- γ A specifically immunoprecipitated the 68 kD disulfide-linked γ , δ TCR heterodimer from the M13.PBL C1 transfectant cells (Fig. 19B, lanes 7 and 8), and the 55 kD γ TCR chain, along with the 40 kD γ TCR chain, from the M13.IDP2 γ transfectant cells (Fig. 19C, lanes 7 and 8), confirming that the γ TCR chains that are part of these complexes correspond to the transfected PBL C1 and IDP2-derived γ TCR cDNAs, respectively.

To further characterize the various γ TCR proteins biochemically, two-dimensional (2D) gel analyses (NEPHGE followed by SDA-PAGE) of surface 125 I-labeled cells were carried out. Superimposition of the 2D patterns of resident (MOLT-13) and transfected (PBL C1 or IDP2) γ TCR chains relative to the positions of the CD3 components allowed comparison of the relevant γ TCR species. In the immunoprecipitates from MOLT-13 cells, the γ TCR chains resolved as two discrete parallel series of iodinated species (Fig. 20A, see open arrows). MOLT-13 cells transfected with the PBL C1 or the IDP2 TCR cDNAs revealed the resident MOLT-13 γ TCR polypeptide series, but in addition, showed radiolabeled species that were identical in 2D gel patterns to the γ TCR polypeptides of PBL C1 (compare Fig. 20B and D; see asterisks in Fig. 20B) or IDP2 (compare Fig. 20C and D; see closed arrows in Fig. 20C) cells, respectively. Thus, the 2D gel biochemical analyses confirmed that the transfected γ TCR chains were expressed and processed similarly in MOLT-13 transfectants and in the parental cell lines, PBL C1 and IDP2.

9.2.3. POLYPEPTIDE BACKBONE SIZES OF THE TRANSFECTED γ TCR CHAIN PROTEINS

The peptide backbone sizes of the transfected γ TCR chains were determined by endoglycosidase-H treatment of the material immunoprecipitated from metabolically pulse-labeled cells. Immunoprecipitates carried out with anti-C γ M1 (specific for γ TCR chain) identified 35.5 and 34 kD species in untransfected MOLT-13 cells (Fig. 21, lane 4) that represent endogenous MOLT-13 γ TCR polypeptides. The smaller of these two polypeptides (see open arrows) corresponds to the expected polypeptide core size of the MOLT-13 γ TCR polypeptide, whereas the larger polypeptide appears to represent a partially processed intermediate. In addition to these resident MOLT-13 γ TCR polypeptides, a polypeptide with a deglycosylated size of 41 kD was immunoprecipitated by anti-C γ M1 from the M13.IDP2 γ transfectant, (Fig. 21, lane 8, see solid arrow). The size of this transfectant-specific γ TCR polypeptide agrees well with the deglycosylated IDP2 γ TCR polypeptide core size determined earlier in IDP2 cells (Brenner, M.B., et al., 1987, Nature 325:689-694). As expected, M13.PBL C1 γ transfectant cells revealed an additional γ TCR protein with a deglycosylated size of 32 kD (Fig. 21, lane 12, see solid arrow) which compares well with the γ TCR polypeptide backbone size reported earlier for the PBL C1 cell line (Brenner, M.B., et al., 1987, Nature 325:689-694). This 32 kD species was specifically immunoprecipitated by the V γ 2-specific mAb, anti-Ti- γ A (Fig. 21, lane 13, see solid arrow), thereby allowing unambiguous assignment of resident and transfected γ TCR species in this cell line. Thus, the determined backbone sizes of the transfected γ TCR chains, derived from IDP2 and PBL C1 cell lines, match the backbone sizes of these polypeptides in their parent cell lines. By comparing the γ TCR polypeptide core sizes with those of the cell surface proteins, we infer that the MOLT-13, IDP2, and PBL C1 derived γ TCR chains carry 6, 14, and 8 kD N-linked carbohydrate, respectively.

9.3. DISCUSSION

Three biochemically distinct forms of the human γ δ TCR subunit structure occur. In the present work, we show that a single δ TCR polypeptide can associate with γ TCR chains representing each of the three receptor forms to reconstitute the appropriate γ , δ TCR heterodimers. The resident γ TCR polypeptide of MOLT-13 (form 2bc) is 40 kD and is noncovalently associated with the δ TCR subunit. When the γ TCR cDNA clones correspond-

ing to the disulfide-linked receptor of PBL C1 (Form 1), or the 55 kD non-disulfide-linked receptor of the IDP2 cell line (Form 2abc) were transfected into the MOLT-13 cell line, the $\gamma\delta$ TCR forms corresponding to those found in the cDNA-donor cell lines were reconstituted. The present transfection studies provide direct evidence that disulfide linkage is dictated by γ TCR constant segment usage, since the resident MOLT-13 δ TCR chain was shown to participate in a disulfide-linked receptor complex with the PBL C1-derived γ TCR chain (Form 1), and a nondisulfide-linked receptor complex with the IDP2-derived γ TCR chain (Form 2abc).

We have shown that the remarkable difference in size between the 55 kD (Form 2abc) and 40 kD (Form 2bc) non-disulfide-linked γ TCR polypeptides is primarily due to different amounts of N-linked carbohydrate attached to the γ TCR polypeptide backbone (See Section 8.2.2, *supra*). Thus, either 15 kD (Form 2abc on IDP2 or PEER) or only 5 kD (Form 2bc on MOLT-13) of N-linked carbohydrate is attached to these γ TCR polypeptides even though the same number (five each) of N-linked glycan acceptor sites are encoded by the constant region gene segments used in both of these forms. Four of these N-linked glycosylation sites are present in or around the CII exon-encoded connector region. In the example herein, we show that the amount of N-linked carbohydrate attached to the transfected γ TCR proteins is identical to that seen in their parent cell lines, based on a comparison of peptide core size and mature cell surface size of the protein products of transfected γ TCR cDNA clones. Thus, the conformation of the two C γ 2 encoded protein segments must differ sufficiently to result in drastic differences in glycosylation. The major difference between C γ segments of these two forms is that copy "a" of the CII exon is present in the 55 kD γ TCR chain of Form 2abc and it is absent from the 40 kD γ TCR chain of Form 2bc. Thus the presence or absence of this CII exon copy may be largely responsible for the glycosylation differences that account for the γ TCR polypeptide sizes.

The variation in structure of human $\gamma\delta$ TCR isotypic forms is unprecedented among T cell receptors as no such parallel is observed in $\alpha\beta$ TCR.

10. T CELL RECEPTOR $\gamma\delta$ COMPLEX, NOT ASSOCIATED WITH CD3, IS IDENTIFIED IN HUMAN ENDOMETRIAL GLANDULAR EPITHELIUM

In the early stages of placentation, infiltration of mononuclear cells is abundant at the proximity of spiral arteries and endometrial glands in maternal uterine tissues. These include an unusual population of T lineage cells of unknown function. Many extravillous trophoblasts express a novel type of class I MHC antigens which is different from that expressed on most somatic cells. We have tested a panel of monoclonal antibodies to TCR $\gamma\delta$ heterodimer ($\gamma\delta$ TCR) in pregnant & non-pregnant uteri. Surprisingly, $\gamma\delta$ TCR complex was not detected in leukocytes, but was localized in the cytoplasm of the endometrial glandular epithelium from pregnant uteri. These antibodies also react with the glandular epithelium from non-pregnant uteri, and the reactivity was stronger in the secretory phase than that in the proliferative phase of the menstrual cycle. However, $\gamma\delta$ TCR was not associated with the CD3 complex, as shown by examining immunoprecipitates using three different monoclonal antibodies to CD3 (OKT3, anti-leu-4, UCHT-1). The $\gamma\delta$ TCR-positive glandular epithelial cells did not react with monoclonal antibodies to $\alpha\beta$ TCR; the cells were also CD4- and CD8-negative. Moreover, the glandular epithelial cells lose the class I MHC antigens in early pregnancy. These data suggest that these $\gamma\delta$ TCR bearing endometrial glandular cells undergo, at least, phenotypic alterations under local regulation of gene expression.

11. EXAMPLE: CHARACTERIZATION OF A HUMAN δ T CELL RECEPTOR GENE AND A V δ SPECIFIC MONOCLONAL ANTIBODY

We have isolated δ TCR cDNA and a rearranged δ TCR gene from a human $\gamma\delta$ T cell clone, AK119. From these DNA clones, a K δ probe was obtained, and used to determine the diversity of δ TCR gene rearrangements in a panel of 13 human γ,δ T cell clones and 3 γ,δ human T cell tumor lines. Altogether five different rearrangements were detected, which corresponded to rearrangements using 2 to 5 different X δ genes. One particular rearrangement was always seen in human γ,δ T cells that reacted with mAb TCS δ 1 (δ TCAR-3). In addition, TCS δ 1 immunoprecipitated with δ TCR polypeptide from a human γ,δ tumor cell line, Molt 13. We provide evidence that monoclonal antibody TCS δ 1 recognizes an epitope encoded in the AK119 V δ gene or in a combination epitope of the rearranged AK119 gene V δ -J δ gene.

11.1. MATERIALS AND METHODS

11.1.1. ISOLATION AND SEQUENCING OF AK119 δ TCR cDNA CLONES

A cDNA library was generated from the PBL T-cell clone, AK119, by the method of Gubler and Hoffmann (Gubler and Hoffman, 1983, Gene 25:263). About 100,000 plaques of an amplified library were screened using

a ^{32}P -labelled nick-translated C δ probe, isolated from a δ TCR clone called O-024 (Hata, S., et al., 1987, Science 238:678). The longest hybridizing cDNA clone (1.3 kb clone C11983) was selected for sequence analysis by the dideoxy chain termination method.

5 11.1.2. CLONING A REARRANGED δ TCR GENE

A 3.5 kb genomic DNA clone containing the rearranged V δ gene was obtained from AK119 cells as follows: EcoRI digested DNA was size fractionated on a preparative agarose gel, ligated into λ gt10, packaged and transfected into E. coli. Recombinant phage were screened with a ^{32}P -labeled nick translated 550 bp EcoRI fragment derived from the cDNA clone, c11983. A rearranged clone called r11981 which contains a 0.8 kb HincII fragment (V region specific) and a 1 kb HincII-EcoRI fragment (V-J region) was isolated.

11.1.3. DNA PREPARATION

15 Fetal and newborn thymic tissues were collected in accordance with accepted guidelines regarding patients' rights and approval. T cell clones were obtained from peripheral blood, pleural exudate or cerebrospinal fluid by limiting dilution and were cultured in vitro (Hafler et al., 1985, Ann Neurol. 18:451; Van de Griend et al., 1987, J. Immunol. 138:1627). In all cases, DNA was prepared by digestion with proteinase K in 1% sodium dodecyl sulfate, followed by extraction with phenol/chloroform and ethanol precipitation.

20 11.1.4 SOUTHERN BLOT ANALYSIS

Genomic DNA was digested with EcoRI, size fractionated on a 0.9% agarose gel and transferred to nitro-cellulose. Hybridization was carried out with ^{32}P -nick translated probes as previously described (Maniatis, 25 1982, Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory; Cold Spring Harbor, New York).

11.1.5 CYTOFLUOROMETRIC ANALYSIS

30 Normal peripheral blood mononuclear cells (PBMC), obtained from volunteers were isolated by fractionation on a Ficoll gradient. PBMC and PBL T cell clones were stained by indirect immunofluorescence using TCS δ 1 mAb (referred to previously as δ TCAR-3; See Section 7, supra) and fluorescein-conjugated goat antimouse IgG (Becton Dickinson) and analyzed in a fluorescence activated flow cytometer.

35 11.2. RESULTS

11.2.1. DIVERSITY OF δ TCR GENE REARRANGEMENTS

40 Using a C δ probe, we isolated a 1.3 kb δ TCR cDNA clone, termed c11983, from a λ gt10 cDNA library of the T-cell clone AK119. The 5' end of c11983 was sequenced and found to use previously identified V δ and J δ genes (Hata et al., 1987, Science 238:678; Loh et al., 1987, Nature 330:569). The sequence of the V-J junction indicated that C11983 has an in-frame V-J joint.

45 A 550 basepair (bp) EcoRI fragment encoding all the variable and joining region and part of the constant region (V-J-C probe) was obtained from c11983 and used in Southern blot analysis of EcoRI digested genomic DNA from AK119. This probe detects a germline 3.2 kb V δ and a germline 1.0 kb C δ band. AK119 showed an extra rearranged 2.5 kb band that is identical to the common δ TCR rearrangement (described in Hata et al., 1987, Science 238:678; Loh et al., 1987, Nature 330:569) (rearrangement II in Fig. 22). This 3.5 kb band was cloned from a EcoRI size-fractionated λ gt10 genomic library using the V-J-C cDNA probe. A partial map of the cloned rearranged δ TCT gene, called r11981, is shown in Figure 17. The localization of the variable and joining region was determined using J oligonucleotide probes and variable region specific probes. From r11981, 50 a 1 kb V-J probe was isolated by digestion with HincII and EcoRI enzyme (see Fig. 17).

This V-J probe was used to determine the diversity of δ TCR gene rearrangements in a panel of 13 human $\gamma\delta$ TCR positive T-cell clones and 3 human $\gamma\delta$ TCR positive tumor cell lines. As shown in Figure 22, five common rearrangements, numbered I-V, are seen in the polyclonal newborn thymocyte sample (lane 11). These rearrangements are representative of rearrangements used by the human $\gamma\delta$ T cell clones. Only rearrangement II hybridizes to the HincII - HincII V δ specific probe. Although we do not know that all these arrangements represent V-D-J rather than D-J rearrangements, some of them must represent rearrangements of new variable regions to the previously characterized J δ gene segment because these cells express a functional δ TCR polypeptide chain on their cell surfaces. We have not ruled out the possibility that these new rearrangements rep-

resent rearrangements of a single new V_δ gene to other J_δ genes, yet to be identified. Our data is consistent with the fact that there must be 2-5 variable region genes that can be used in δ TCR gene rearrangements.

11.2.2. DETERMINING THE SPECIFICITY OF mAb TCS δ 1

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TCS δ 1 previously referred to as δ TCAR-3, was generated by fusing splenocytes from mice immunized with the human tumor $\gamma\delta$ TCR cell line MOLT-13 with a mouse myeloma line. When used in fluorescent activated cell sorter analysis, TCS δ 1 reacted only with some but not all human $\gamma\delta$ T cells. The results are given in Table 1. There is a perfect correlation with usage of the AK119 V_δ gene (rearrangement II) with positive staining by TCS δ 1. This data provides strong evidence that the epitope recognized by δ TCS1 is encoded in the AK119 V_δ gene or in a combinatorial epitope of the rearranged AK119 V_δ - J_δ gene.

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TABLE 1
CORRELATION BETWEEN STAINING BY TCS1 δ mAb AND
A SPECIFIC V δ REARRANGEMENT

	<u>human $\gamma\delta$TCR T cell clones</u>	<u>δ rearrangement¹</u>	<u>TCSδ1</u>
10	AK4	V/? ²	- ⁴
	AK615	I/IV	-
	AK925	III/?	nd
	1004	I/IV	-
15	1005	I/IV	-
	1011	I/IV	-
	1012	I/IV	-
20	1015	I/?	-
	1018	IV/?	-
	Wi.1	I/?	-
	1019	II/IV	nd
25	AK119	II/?	+
	Wi.K	II/III	+
	<u>human $\gamma\delta$TCR T cell tumor lines</u>		
30	Peer	II/?	+
	Molt-13	II/V	+
	DND41	II/VI ³	+

- 1 δ TCR rearrangements detected with V-J probe, numbered I-V as in Figure 22.
- 2 Only 1 rearrangement was identified in each case even though no germline J δ was detected.
- 3 A new rearrangement is observed which is not seen in newborn or fetal thymocytes. This rearrangement has been assigned rearrangement VI.
- 4 + means positive staining, - means negative staining. nd means not determined.

12. DEPOSIT OF HYBRIDOMAS

The following hybridoma cell lines, producing the indicated monoclonal antibody, have been deposited with the American Type Culture Collection (ATCC), 12310 Parklawn Drive, Rockville, Maryland 20852, U.S.A. on the indicated dates, and have been assigned the listed accession numbers:

	<u>Hybridoma</u>	<u>Monoclonal Antibody</u>	<u>Date of Deposit</u>	<u>Accession Number</u>
5	δ TCAR-3	TCS δ 1 (δ TCAR-3) (anti-V δ)	10/29/87	HB 9578
	5A6.E9	anti-TCR δ 1 (anti-C δ)	7/27/88	HB 9772
10	#3	anti-C γ m1 (anti-C γ)	7/27/88	HB 9773

The following hybridoma cell lines have also been deposited with the American Type Culture Collection, on the indicated dates, and have been assigned the listed accession numbers:

	<u>Hybridoma</u>	<u>Date of Deposit</u>	<u>Accession Number</u>
15			
20	R2B7	9/28/88	HB 9842
	8F4	9/28/88	HB 9843

The present invention is not to be limited in scope by the cell lines deposited since the deposited embodiments are intended as single illustrations of one aspect of the invention and any cell lines which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

Claims

1. A monoclonal antibody reactive with an epitope of a variable or rearranged variable-joining region of a δ chain of the human T cell antigen receptor.
2. The monoclonal antibody of claim 1 which comprises monoclonal antibody TCS δ 1 (δ TCAR-3) as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9578.
3. A monoclonal antibody reactive with an epitope of the constant region of the δ chain of the human T cell antigen receptor.
4. The monoclonal antibody of claim 3 which comprises monoclonal antibody anti-TCR δ 1 as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9772.
5. A monoclonal antibody reactive with an epitope of the constant region of the Form 2bc gamma chain of the human T cell antigen receptor, which epitope is characteristic of the Form 2bc gamma chain.
6. A monoclonal antibody anti-C γ m1 as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9773.
7. The Fv, Fab, Fab', or F(ab')₂ fragment of the monoclonal antibody of claim 1, 2 or 3.
8. The Fv, Fab, Fab', or F(ab')₂ fragment of the monoclonal antibody of claim 4, 5 or 6.
9. A hybridoma producing the monoclonal antibody of claim 1 or 2.
10. A hybridoma producing the monoclonal antibody of claim 3 or 4.

	<u>Hybridoma</u>	<u>Monoclonal Antibody</u>	<u>Date of Deposit</u>	<u>Accession Number</u>
5	δ TCAR-3	TCS δ 1 (δ TCAR-3) (anti-V δ)	10/29/87	HB 9578
	5A6.E9	anti-TCR δ 1 (anti-C δ)	7/27/88	HB 9772
10	#3	anti-C γ m1 (anti-C γ)	7/27/88	HB 9773

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15

	<u>Hybridoma</u>	<u>Date of Deposit</u>	<u>Accession Number</u>
20	R2B7	9/28/88	HB 9842
	8F4	9/28/88	HB 9843

The present invention is not to be limited in scope by the cell lines deposited since the deposited embodiments are intended as single illustrations of one aspect of the invention and any cell lines which are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

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Claims

1. A monoclonal antibody reactive with an epitope of a variable or rearranged variable-joining region of a δ chain of the human T cell antigen receptor.
2. The monoclonal antibody of claim 1 which comprises monoclonal antibody TCS δ 1 (δ TCAR-3) as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9578.
3. A monoclonal antibody reactive with an epitope of the constant region of the δ chain of the human T cell antigen receptor.
4. The monoclonal antibody of claim 3 which comprises monoclonal antibody anti-TCR δ 1 as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9772.
5. A monoclonal antibody reactive with an epitope of the constant region of the Form 2bc gamma chain of the human T cell antigen receptor, which epitope is characteristic of the Form 2bc gamma chain.
6. A monoclonal antibody anti-C γ m1 as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9773.
7. The Fv, Fab, Fab', or F(ab')₂ fragment of the monoclonal antibody of claim 1, 2 or 3.
8. The Fv, Fab, Fab', or F(ab')₂ fragment of the monoclonal antibody of claim 4, 5 or 6.
9. A hybridoma producing the monoclonal antibody of claim 1 or 2.
10. A hybridoma producing the monoclonal antibody of claim 3 or 4.



11. A hybridoma producing the monoclonal antibody of claim 5 or 6.
12. A method for detecting the presence of a functional rearrangement of a human δ T cell antigen receptor variable gene in a cell which comprises contacting the cell or a sample containing polypeptides of the cell with a monoclonal antibody specifically reactive with an epitope of a polypeptide encoded by the rearranged gene.
13. The method according to claim 12 in which the monoclonal antibody comprises TCS δ 1 (δ TCAR-3) as produced by the hybridoma deposited with the ATCC and assigned accession number HB 9578.
14. The monoclonal antibody of claim 1 which is characterized by the ability to co-modulate a CD3 antigen.
15. The monoclonal antibody of claim 3 which is characterized by the ability to co-modulate a CD3 antigen.
16. The monoclonal antibody of claim 5 which is characterized by the ability to co-modulate a CD3 antigen.
17. A purified polypeptide comprising an amino acid sequence of a constant region of a gamma T cell antigen receptor Form 2bc encoded by nucleotide numbers 439 through 1008 of Figure 14.
18. The purified polypeptide of claim 17, which comprises a gamma T cell antigen receptor Form 2bc, having a molecular weight of about 40,000 daltons and a sequence comprising a constant region consisting essentially of the amino acid sequence encoded by nucleotide numbers 439 through 1008 of Figure 14, and a variable region.
19. A purified polypeptide comprising a gamma T cell antigen receptor Form 2bc having a primary amino acid sequence substantially as depicted in Figure 14 for the variable, N, joining, and constant regions.
20. An isolated nucleic acid encoding the polypeptide of claim 17.
21. An isolated nucleic acid encoding the polypeptide of claim 18.
22. An isolated nucleic acid encoding the polypeptide of claim 19.
23. The nucleic acid of claim 22 which is a cDNA clone.
24. A nucleic acid vector comprising the cDNA clone of claim 23.
25. A cell containing the nucleic acid vector of claim 24.
26. The isolated nucleic acid of claim 20, 21 or 22 which comprises DNA.
27. The isolated nucleic acid of claim 20, 21 or 22 which comprises RNA.
28. A purified polypeptide complex comprising a T cell antigen receptor heterodimer consisting of the gamma T cell antigen receptor polypeptide of claim 17 and a second T cell antigen receptor polypeptide selected from the group consisting of the α , β , gamma, and δ T cell antigen receptor polypeptides.
29. The complex of claim 28 in which the second T cell antigen receptor is the δ T cell antigen receptor polypeptide.
30. A method for producing a gamma,delta T cell antigen receptor heterodimer which comprises culturing a cell (a) capable of expressing a nucleic acid encoding a δ T cell antigen receptor polypeptide, and (b) which has been transfected with a nucleic acid encoding a gamma T cell antigen receptor polypeptide, under conditions such that both the gamma T cell antigen receptor polypeptide and the δ T cell antigen receptor polypeptide are expressed by the cell and assemble to form a heterodimer.
31. The method according to claim 30 in which the gamma T cell antigen receptor polypeptide comprises an amino acid sequence of a constant region of a gamma T cell antigen receptor polypeptide Form 2bc encoded by nucleotide numbers 439 through 1008 of Figure 14.
32. The method according to claim 30 in which the gamma T cell antigen receptor polypeptide is Form 2bc



having a primary amino acid sequence substantially as depicted in Figure 14 for the variable, N, joining, and constant regions.

- 5 33. The method according to claim 30 in which the gamma T cell antigen receptor polypeptide is Form 2bc having a molecular weight of about 40,000.
34. The method according to claim 30 in which the subunits of the heterodimer are disulfide-linked, and the gamma T cell antigen receptor polypeptide is Form 1 having a molecular weight of about 40,000.
- 10 35. The method according to claim 30 in which the gamma T cell antigen receptor polypeptide is Form 2abc having a molecular weight of about 55,000 daltons.
36. The method according to claim 30 in which the cell is a T cell.
- 15 37. The method according to claim 30 in which the nucleic acid encoding a gamma T cell antigen receptor polypeptide is selected from the group consisting of (i) a nucleic acid comprising a single CII exon; (ii) a nucleic acid comprising two CII exons; and (iii) a nucleic acid comprising three CII exons.
38. The method according to claim 30 in which the expressed gamma T cell antigen receptor polypeptide and the expressed δ T cell antigen receptor polypeptide are non-covalently associated.
- 20 39. The method according to claim 30 in which the expressed gamma T cell antigen receptor polypeptide and the expressed δ T cell antigen receptor polypeptide are covalently associated.
- 25 40. A method for producing at least a portion of a gamma,delta T cell antigen receptor heterodimer, which comprises culturing a transfected cell (a) capable of expressing a nucleic acid encoding at least a region of a gamma T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, and a joining region; and (b) capable of expressing a nucleic acid encoding at least a region of a δ T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, a joining region, and a diversity region; and subjecting the cell to conditions such that both nucleic acid sequences are expressed by the cell.
- 30 41. A method for expressing at least a portion of a gamma,delta T cell antigen receptor heterodimer, which comprises culturing a transfected cell (a) capable of expressing a nucleic acid encoding at least a region of a gamma T cell antigen receptor polypeptide selected from the group consisting of a variable region, a joining region, and a constant region; and (b) capable of expressing a nucleic acid encoding at least a region of a δ T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, a joining region, and a diversity region; and subjecting the cell to conditions such that both nucleic acid sequences are expressed by the cell, in which the nucleic acid encoding at least a region of the gamma T cell antigen receptor polypeptide was transfected into the cell.
- 35 42. The method according to claim 41 in which the gamma T cell antigen receptor polypeptide comprises Form 2bc, having a molecular weight of about 40,000 daltons and a sequence comprising a constant region consisting essentially of the amino acid sequence encoded by nucleotide numbers 439 through 1008 of Figure 14, and a variable region.
- 40 43. The method according to claim 40, 41 or 42, in which the cell is a T cell.
- 45 44. The method according to claim 40 or 41 in which the nucleic acid encoding at least a portion of the gamma T cell antigen receptor polypeptide is selected from the group consisting of (i) a nucleic acid comprising a single CII exon; (ii) a nucleic acid comprising two CII exons; and (iii) a nucleic acid comprising three CII exons.
- 50 45. The method according to claim 40 or 41 in which the portion of the gamma T cell antigen receptor polypeptide and the portion of the δ T cell antigen receptor polypeptide are non-covalently associated.
- 55 46. The method according to claim 40 or 41 in which the portion of the gamma T cell antigen receptor polypeptide and the portion of the δ T cell antigen receptor polypeptide are covalently associated.
47. Isolated cells which express at least a portion of a gamma, delta T cell antigen receptor heterodimer, which



portion of a gamma,delta T cell antigen receptor comprises at least a region of a gamma T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, and a joining region, associated with at least a region of a δ T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, a joining region, and a diversity region; which heterodimer is not associated with a CD3 complex.

48. The cells of claim 47 in which the cells are endometrial glandular epithelial cells.

49. A transfected cell which expresses a gamma,delta T cell antigen receptor heterodimer, in which the gamma T cell antigen receptor polypeptide of the heterodimer is produced by expression of a nucleic acid encoding the gamma T cell antigen receptor polypeptide which was transfected into the cell.

50. The cell of claim 49 in which the gamma T cell antigen receptor polypeptide is Form 2bc having a molecular weight of about 40,000 daltons and a sequence comprising a constant region consisting essentially of the amino acid sequence encoded by nucleotide numbers 439 through 1008 of Figure 14, and a variable region.

51. A transfected cell which expresses at least a portion of a gamma,delta T cell antigen receptor heterodimer, which portion of a gamma,delta T cell antigen receptor comprises at least a region of a gamma T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, and a joining region, associated with at least a region of a δ T cell antigen receptor polypeptide selected from the group consisting of a constant region, a variable region, a joining region, and a diversity region; which heterodimer is not associated with a CD3 complex, in which the portion of the gamma T cell antigen receptor polypeptide is produced by expression of a nucleic acid encoding the portion of the gamma T cell antigen receptor which was transfected into the cell.

52. A method for determining the relative usage of a gamma T cell antigen receptor polypeptide in a sample from a human subject comprising:

- (a) determining in a sample containing T cells from a subject the amount of a gamma Form 2bc T cell antigen receptor polypeptide;
- (b) determining in a sample from a subject the amount of a gamma T cell antigen receptor polypeptide selected from the group consisting of a Form 1 gamma polypeptide, and Form 2abc gamma polypeptide; and
- (c) comparing the amount determined in step (a) with the amount determined in step (b), thereby determining the relative usage.

Patentansprüche

1. Monoklonaler Antikörper, der gegenüber einem Epitop einer variablen oder rearrangierten variablen-verknüpfenden (joining) Region einer δ -Kette des menschlichen T-Zell-Antigen-Rezeptors reaktiv ist.
2. Monoklonaler Antikörper nach Anspruch 1, umfassend den monoklonalen Antikörper TCS δ 1 (δ TCAR-3), wie er von dem Hybridom produziert wird, welches beim ATCC unter der Zugriffsnummer HB 9578 hinterlegt ist.
3. Monoklonaler Antikörper, der gegenüber einem Epitop der konstanten Region der δ -Kette des menschlichen T-Zell-Antigen-Rezeptors reaktiv ist.
4. Monoklonaler Antikörper nach Anspruch 3, umfassend den monoklonalen Antikörper Anti-TCR δ 1, wie er von dem Hybridom produziert wird, welches beim ATCC unter der Zugriffsnummer HB 9772 hinterlegt ist.
5. Monoklonaler Antikörper, der gegenüber einem Epitop der konstanten Region der Form 2bc- γ -Kette des menschlichen T-Zell-Antigen-Rezeptors reaktiv ist, wobei das Epitop für die Form 2bc- γ -Kette charakteristisch ist.
6. Monoklonaler Antikörper Anti-Cyml, wie er von dem Hybridom produziert wird, welches beim ATCC unter der Zugriffsnummer HB 9773 hinterlegt ist.



7. Fv-, Fab-, Fab'- oder F(ab')₂-Fragment des monoklonalen Antikörpers gemäß Anspruch 1, 2 oder 3.
8. Fv-, Fab-, Fab'- oder F(ab')₂-Fragment des monoklonalen Antikörpers gemäß Anspruch 4, 5 oder 6.
- 5 9. Hybridom, das den monoklonalen Antikörper gemäß Anspruch 1 oder 2 produziert.
10. Hybridom, das den monoklonalen Antikörper gemäß Anspruch 3 oder 4 produziert.
11. Hybridom, das den monoklonalen Antikörper gemäß Anspruch 5 oder 6 produziert.
- 10 12. Verfahren zum Nachweis des Vorliegens eines funktionellen Rearrangements eines menschlichen δ -T-Zell-Antigen-Rezeptorvariablen Gens in einer Zelle, umfassend das Kontaktieren der Zelle oder einer Probe, die Polypeptide der Zelle enthält, mit einem monoklonalen Antikörper, der in spezifischer Weise gegenüber einem Epitop eines von dem rearrangierten Gen kodierten Polypeptids reaktiv ist.
- 15 13. Verfahren nach Anspruch 12, bei dem der monoklonale Antikörper TCS δ 1 (δ TCAR-3) umfaßt, wie er von dem Hybridom produziert wird, welches beim ATCC unter der Zugriffsnummer HB 9578 hinterlegt ist.
14. Monoklonaler Antikörper nach Anspruch 1, der durch die Fähigkeit gekennzeichnet ist, ein CD3-Antigen zu comodulieren.
- 20 15. Monoklonaler Antikörper nach Anspruch 3, der durch die Fähigkeit gekennzeichnet ist, ein CD3-Antigen zu comodulieren.
16. Monoklonaler Antikörper nach Anspruch 5, der durch die Fähigkeit gekennzeichnet ist, ein CD3-Antigen zu comodulieren.
- 25 17. Gereinigtes Polypeptid, das eine Aminosäuresequenz einer konstanten Region eines γ -T-Zell-Antigen-Rezeptors Form 2bc umfaßt, die durch die Nukleotide 439 bis 1008 der Figur 14 kodiert wird.
- 30 18. Gereinigtes Polypeptid nach Anspruch 17, umfassend einen γ -T-Zell-Antigen-Rezeptor Form 2bc mit einer relativen Molekülmasse von etwa 40 000 Dalton und einer Sequenz, die eine konstante Region umfaßt, welche im wesentlichen aus der Aminosäuresequenz besteht, welche durch die Nukleotide 439 bis 1008 der Figur 14 kodiert wird, und eine variable Region.
- 35 19. Gereinigtes Polypeptid, umfassend einen γ -T-Zell-Antigen-Rezeptor Form 2bc mit einer primären Aminosäuresequenz im wesentlichen gemäß Darstellung in Figur 14 für die variable, N-, verknüpfende (joining) und konstante Region.
20. Isolierte Nukleinsäure, die für das Polypeptid gemäß Anspruch 17 kodiert.
- 40 21. Isolierte Nukleinsäure, die für das Polypeptid gemäß Anspruch 18 kodiert.
22. Isolierte Nukleinsäure, die für das Polypeptid gemäß Anspruch 19 kodiert.
23. Nukleinsäure nach Anspruch 22, welche ein cDNA-Klon ist.
- 45 24. Nukleinsäurevektor, umfassend den cDNA-Klon gemäß Anspruch 23.
25. Zelle, die den Nukleinsäurevektor gemäß Anspruch 24 enthält.
26. Isolierte Nukleinsäure nach Anspruch 20, 21 oder 22, die DNA umfaßt.
- 50 27. Isolierte Nukleinsäure nach Anspruch 20, 21 oder 22, die RNA umfaßt.
28. Gereinigter Polypeptidkomplex, umfassend ein T-Zell-Antigen-Rezeptor-Heterodimer, bestehend aus dem γ -T-Zell-Antigen-Rezeptor-Polypeptid gemäß Anspruch 17 und einem zweiten T-Zell-Antigen-Rezeptor-Polypeptid, ausgewählt aus der Gruppe bestehend aus den α -, β -, γ - und δ -T-Zell-Antigen-Rezeptor-Polypeptiden.
- 55 29. Komplex nach Anspruch 28, bei dem der zweite T-Zell-Antigen-Rezeptor das δ -T-Zell-Antigen-Rezeptor-



Polypeptid ist.

- 5 30. Verfahren zur Herstellung eines γ,δ -T-Zell-Antigen-Rezeptor-Heterodimers, umfassend das Kultivieren einer Zelle, die (a) in der Lage ist, eine Nukleinsäure zu exprimieren, welche für ein δ -T-Zell-Antigen-Rezeptor-polypeptid kodiert, und die (b) mit einer Nukleinsäure transfiziert worden ist, welche für ein γ -T-Zell-Antigen-Rezeptor-Polypeptid kodiert, unter solchen Bedingungen, daß sowohl das γ -T-Zell-Antigen-Rezeptor-Polypeptid als auch das δ -T-Zell-Antigen-Rezeptor-Polypeptid von der Zelle exprimiert werden und sich zur Bildung eines Heterodimers zusammenfügen.
- 10 31. Verfahren nach Anspruch 30, bei dem das γ -T-Zell-Antigen-Rezeptor-Polypeptid eine Aminosäuresequenz einer konstanten Region eines γ -T-Zell-Antigen-Rezeptor-Polypeptids Form 2bc umfaßt, welche durch die Nukleotide 439 bis 1008 der Figur 14 kodiert wird.
- 15 32. Verfahren nach Anspruch 30, bei dem das γ -T-Zell-Antigen-Rezeptor-Polypeptid Form 2bc ist mit einer primären Aminosäuresequenz im wesentlichen gemäß Darlegung in Figur 14 für die variable, N-, verknüpfende (joining) und konstante Region.
- 20 33. Verfahren nach Anspruch 30, bei dem das γ -T-Zell-Antigen-Rezeptor-Polypeptid Form 2bc ist mit einer relativen Molekülmasse von etwa 40 000.
- 25 34. Verfahren nach Anspruch 30, bei dem die Untereinheiten des Heterodimers über Disulfidbrücken verknüpft sind, und bei dem das γ -T-Zell-Antigen-Rezeptor-Polypeptid Form 1 ist mit einer relativen Molekülmasse von etwa 40 000.
- 30 35. Verfahren nach Anspruch 30, bei dem das γ -T-Zell-Antigen-Rezeptor-Polypeptid Form 2abc ist mit einer relativen Molekülmasse von etwa 55 000 Dalton.
- 35 36. Verfahren nach Anspruch 30, bei dem die Zelle eine T-Zelle ist.
37. Verfahren nach Anspruch 30, bei dem die Nukleinsäure, die für ein γ -T-Zell-Antigen-Rezeptor-Polypeptid kodiert, ausgewählt wird aus der Gruppe bestehend aus: (i) einer Nukleinsäure, die ein einziges CII-Exon umfaßt; (ii) einer Nukleinsäure, die zwei CII-Exons umfaßt; und (iii) einer Nukleinsäure, die drei CII-Exons umfaßt.
38. Verfahren nach Anspruch 30, bei dem das exprimierte γ -T-Zell-Antigen-Rezeptor-Polypeptid und das exprimierte δ -T-Zell-Antigen-Rezeptor-Polypeptid nicht-kovalent assoziiert sind.
39. Verfahren nach Anspruch 30, bei dem das exprimierte γ -T-Zell-Antigen-Rezeptor-Polypeptid und das exprimierte δ -T-Zell-Antigen-Rezeptor-Polypeptid kovalent assoziiert sind.
- 40 40. Verfahren zur Herstellung mindestens eines Teils eines γ,δ -T-Zell-Antigen-Rezeptor-Heterodimers, umfassend das Kultivieren einer transfizierten Zelle, die (a) in der Lage ist, eine Nukleinsäure zu exprimieren, welche für mindestens eine Region eines γ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, und einer verknüpfenden (joining) Region, kodiert; und die (b) in der Lage ist, eine Nukleinsäure zu exprimieren, welche für mindestens eine Region eines δ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, einer verknüpfenden (joining) Region, und einer Verschiedenheits (diversity)-Region, kodiert; und das Zuführen der Zelle unter solche Bedingungen, daß beide Nukleinsäuresequenzen von der Zelle exprimiert werden.
- 45 41. Verfahren zur Expression mindestens eines Bereiches eines γ,δ -T-Zell-Antigen-Rezeptor-Heterodimers, umfassend das Kultivieren einer transfizierten Zelle, welche für (a) in der Lage ist, eine Nukleinsäure zu exprimieren, die mindestens eine Region eines γ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer variablen Region, einer verknüpfenden (joining) Region, und einer konstanten Region, kodiert; und die (b) in der Lage ist, eine Nukleinsäure zu exprimieren, welche für mindestens eine Region eines δ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, einer verknüpfenden (joining) Region, und einer Verschiedenheits- (diversity) Region, kodiert; und das Zuführen der Zelle unter solche Bedingungen, daß beide Nukleinsäuresequenzen von der Zelle exprimiert werden, wobei die Zelle mit der Nukleinsäure transfiziert worden ist, welche für mindestens eine Region des γ -T-Zell-Antigen-Rezeptor-Polypeptids ko-
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diert.

42. Verfahren nach Anspruch 41, bei dem das γ -T-Zell-Antigen-Rezeptor-Polypeptid Form 2bc umfaßt, eine relative Molekülmasse von etwa 40 000 Dalton aufweist und eine Sequenz besitzt, die eine konstante Region, welche im wesentlichen aus der von den Nukleotiden 439 bis 1008 der Figur 14 kodierten Aminosäuresequenz besteht, und eine variable Region umfaßt.
43. Verfahren nach Anspruch 40, 41 oder 42, bei dem die Zelle eine T-Zelle ist.
44. Verfahren nach Anspruch 40 oder 41, bei dem die Nukleinsäure, welche für mindestens einen Bereich des γ -T-Zell-Antigen-Rezeptor-Polypeptids kodiert, ausgewählt wird aus der Gruppe bestehend aus: (i) einer Nukleinsäure, die ein einziges CII-Exon umfaßt; (ii) einer Nukleinsäure, die zwei CII-Exons umfaßt; und (iii) einer Nukleinsäure, die drei CII-Exons umfaßt.
45. Verfahren nach Anspruch 40 oder 41, bei dem der Bereich des γ -T-Zell-Antigen-Rezeptor-Polypeptids und der Bereich des δ -T-Zell-Antigen-Rezeptor-Polypeptids nicht-kovalent assoziiert sind.
46. Verfahren nach Anspruch 40 oder 41, bei dem der Bereich des γ -T-Zell-Antigen-Rezeptor-Polypeptids und der Bereich des δ -T-Zell-Antigen-Rezeptor-Polypeptids kovalent assoziiert sind.
47. Isolierte Zellen, die mindestens einen Bereich eines γ , δ -T-Zell-Antigen-Rezeptor-Heterodimers exprimieren, wobei der Bereich eines γ , δ -T-Zell-Antigen-Rezeptors mindestens eine Region eines γ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, und einer verknüpfenden (joining) Region, assoziiert mit mindestens einer Region eines δ -T-Zell-Antigen-Rezeptor-Polypeptids ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, einer verknüpfenden (joining) Region, und einer Verschiedenheits (diversity)-Region, umfaßt, und wobei das Heterodimer nicht mit einem CD3-Komplex assoziiert ist.
48. Zellen nach Anspruch 47, wobei die Zellen Drüsenzellen des Endometriomepithels sind.
49. Transfizierte Zelle, die ein γ , δ -T-Zell-Antigen-Rezeptor-Heterodimer exprimiert, wobei das γ -T-Zell-Antigen-Rezeptor-Polypeptid des Heterodimers durch Expression einer Nukleinsäure produziert wird, welche für das γ -T-Zell-Antigen-Rezeptor-Polypeptid kodiert und mit der die Zelle transfiziert worden ist.
50. Zelle nach Anspruch 49, in der das γ -T-Zell-Antigen-Rezeptor-Polypeptid Form 2bc ist mit einer relativen Molekülmasse von etwa 40 000 Dalton und einer Sequenz, die eine konstante Region, welche im wesentlichen aus der von den Nukleotiden 439 bis 1008 der Figur 14 kodierten Aminosäuresequenz besteht, und eine variable Region umfaßt.
51. Transfizierte Zelle, die mindestens einen Bereich eines γ , δ -T-Zell-Antigen-Rezeptor-Heterodimers exprimiert, wobei der Bereich eines γ , δ -T-Zell-Antigen-Rezeptors mindestens eine Region eines γ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, und einer verknüpfenden (joining) Region, assoziiert mit mindestens einer Region eines δ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einer konstanten Region, einer variablen Region, einer verknüpfenden (joining) Region, und einer Verschiedenheits (diversity)-Region, umfaßt; wobei das Heterodimer nicht mit einem CD3-Komplex assoziiert ist, und wobei der Bereich des γ -T-Zell-Antigen-Rezeptor-Polypeptids durch Expression einer Nukleinsäure produziert wird, welche für den Bereich des γ -T-Zell-Antigen-Rezeptors kodiert und mit der die Zelle transfiziert worden ist.
52. Verfahren zur Bestimmung der relativen Verwendung eines γ -T-Zell-Antigen-Rezeptor-Polypeptids in einer Probe aus einem menschlichen Individuum, bei dem man:
 - (a) in einer Probe, die T-Zellen von einem Individuum enthält, die Menge eines γ -Form 2bc-T-Zell-Antigen-Rezeptor-Polypeptids bestimmt;
 - (b) in einer Probe eines Individuums die Menge eines γ -T-Zell-Antigen-Rezeptor-Polypeptids, ausgewählt aus der Gruppe bestehend aus einem Form 1- γ -Polypeptid und einem Form 2abc- γ -Polypeptid, bestimmt; und
 - (c) die in Stufe (a) ermittelte Menge mit der in Stufe (b) ermittelten Menge vergleicht und dadurch die relative Verwendung bestimmt.



Revendications

1. Anticorps monoclonal réactif avec un épitope d'une région variable ou d'une région de jonction variable réarrangée d'une chaîne δ du récepteur d'antigènes de cellules T humaines.
2. Anticorps monoclonal selon la revendication 1, comprenant un anticorps monoclonal TCS δ 1 (δ TCAR-3) tel que produit par l'hybridome déposé à l'ATCC et ayant reçu le numéro d'accès HB 9578.
3. Anticorps monoclonal réactif avec un épitope de la région constante de la chaîne δ du récepteur d'antigènes de cellules T humaines.
4. Anticorps monoclonal selon la revendication 3, comprenant un anticorps monoclonal anti-TCR δ 1 tel que produit par l'hybridome déposé à l'ATCC et ayant reçu le numéro d'accès HB 9772.
5. Anticorps monoclonal réactif avec un épitope de la région constante de la chaîne gamma de Forme 2bc du récepteur d'antigènes de cellules T humaines, lequel épitope est caractéristique de la chaîne gamma de Forme 2bc.
6. Anticorps monoclonal anti-Cym1 tel que produit par l'hybridome déposé à l'ATCC et ayant reçu le numéro d'accès HB 9773.
7. Fragment Fv, Fab, Fab' ou F(ab')₂ de l'anticorps monoclonal selon l'une quelconque des revendications 1, 2 ou 3.
8. Fragment Fv, Fab, Fab' ou F(ab')₂ de l'anticorps monoclonal selon l'une quelconque des revendications 4, 5 ou 6.
9. Hybridome produisant l'anticorps monoclonal selon l'une des revendications 1 ou 2.
10. Hybridome produisant l'anticorps monoclonal selon l'une des revendications 3 ou 4.
11. Hybridome produisant l'anticorps monoclonal selon l'une des revendications 5 ou 6.
12. Procédé pour la détection de la présence d'un réarrangement fonctionnel d'un gène variable de récepteur d'antigènes de cellules T δ humaines dans une cellule, comprenant la mise en contact de la cellule ou d'un échantillon contenant des polypeptides de la cellule avec un anticorps monoclonal spécifiquement réactif avec un épitope d'un polypeptide codé par le gène réarrangé.
13. Procédé selon la revendication 12, dans lequel l'anticorps monoclonal comprend TCS δ 1 (δ TCAR-3) tel que produit par l'hybridome déposé à l'ATCC et ayant reçu le numéro d'accès HB 9578.
14. Anticorps monoclonal selon la revendication 1, caractérisé par son aptitude à co-moduler un antigène CD3.
15. Anticorps monoclonal selon la revendication 3, caractérisé par son aptitude à co-moduler un antigène CD3.
16. Anticorps monoclonal selon la revendication 5, caractérisé par son aptitude à co-moduler un antigène CD3.
17. Polypeptide purifié comprenant une séquence d'acides aminés d'une région constante d'un récepteur d'antigènes de cellules T gamma de Forme 2bc codée par les nucléotides numéros 439 à 1008 de la figure 14.
18. Polypeptide purifié selon la revendication 17, comprenant un récepteur d'antigènes de cellules T gamma de Forme 2bc, ayant une masse moléculaire d'environ 40.000 daltons et une séquence comprenant une région constante consistant essentiellement en la séquence d'acides aminés codée par les nucléotides numéros 439 à 1008 de la figure 14, et une région variable.
19. Polypeptide purifié comprenant un récepteur d'antigènes de cellules T gamma de Forme 2bc ayant une séquence d'acides aminés primaire substantiellement telle que décrite dans la figure 14 pour les régions



variable, N, de jonction et constante.

20. Acide nucléique isolé codant pour le polypeptide selon la revendication 17.
- 5 21. Acide nucléique isolé codant pour le polypeptide selon la revendication 18.
22. Acide nucléique isolé codant pour le polypeptide selon la revendication 19.
23. Acide nucléique selon la revendication 22, qui est un clone d'ADNc.
- 10 24. Vecteur d'acide nucléique, comprenant le clone d'ADNc selon la revendication 23.
25. Cellule contenant le vecteur d'acide nucléique selon la revendication 24.
26. Acide nucléique isolé selon l'une quelconque des revendications 20, 21 ou 22, comprenant de l'ADN.
- 15 27. Acide nucléique isolé selon l'une quelconque des revendications 20, 21 ou 22, comprenant de l'ARN.
28. Complexe polypeptidique purifié, comprenant un hétérodimère de récepteur d'antigènes de cellules T consistant en le polypeptide récepteur d'antigènes de cellules T gamma selon la revendication 17 et un second polypeptide récepteur d'antigènes de cellules T choisi dans le groupe comprenant les polypeptides récepteurs d'antigènes de cellules T α , β , gamma et δ .
- 20 29. Complexe selon la revendication 28, dans lequel le second récepteur d'antigènes de cellules T est le polypeptide récepteur d'antigènes de cellules T δ .
- 25 30. Procédé pour la production d'un hétérodimère de récepteur d'antigènes de cellules T gamma, delta, comprenant la mise en culture d'une cellule (a) apte à exprimer un acide nucléique codant pour un polypeptide récepteur d'antigènes de cellules T δ , et (b) qui a été transfectée avec un acide nucléique codant pour un polypeptide récepteur d'antigènes de cellules T gamma, dans des conditions telles qu'à la fois le polypeptide récepteur d'antigènes de cellules T gamma et le polypeptide récepteur d'antigènes de cellules T δ sont exprimés par la cellule et s'assemblent pour former un hétérodimère.
- 30 31. Procédé selon la revendication 30, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma comprend une séquence d'acides aminés d'une région constante d'un polypeptide récepteur d'antigène de cellules T gamma de Forme 2bc codé par les nucléotides numéros 439 à 1008 de la figure 14.
- 35 32. Procédé selon la revendication 30, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma est la Forme 2bc ayant une séquence d'acides aminés primaire substantiellement telle que décrite dans la figure 14 pour les régions variable, N, de jonction et constante.
- 40 33. Procédé selon la revendication 30, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma est la Forme 2bc ayant une masse moléculaire d'environ 40.000.
- 45 34. Procédé selon la revendication 30, dans lequel les sous-unités de l'hétérodimère sont liées par disulfure, et le polypeptide récepteur d'antigènes de cellules T gamma est la Forme 1 ayant une masse moléculaire d'environ 40.000.
- 50 35. Procédé selon la revendication 30, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma est la Forme 2abc ayant une masse moléculaire d'environ 55.000 daltons.
- 55 36. Procédé selon la revendication 30, dans lequel la cellule est une cellule T.
37. Procédé selon la revendication 30, dans lequel l'acide nucléique codant pour un polypeptide récepteur d'antigènes de cellules T gamma est choisi dans le groupe consistant en (i) un acide nucléique comprenant un unique exon CII; (ii) un acide nucléique comprenant deux exons CII; et (iii) un acide nucléique comprenant trois exons CII.
38. Procédé selon la revendication 30, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma exprimé et le polypeptide récepteur d'antigènes de cellules T δ exprimé ne sont pas associés par covalence.



39. Procédé selon la revendication 30, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma exprimé et le polypeptide récepteur d'antigènes de cellules T δ exprimé sont associés par covalence.
- 5 40. Procédé pour la production d'au moins une portion d'un hétérodimère de récepteur d'antigènes de cellules T gamma,delta, comprenant la mise en culture d'une cellule transfectée (a) apte à exprimer un acide nucléique codant pour au moins une région d'un polypeptide récepteur d'antigènes de cellules T gamma choisie dans le groupe consistant en une région constante, une région variable et une région de jonction; et (b) apte à exprimer un acide nucléique codant pour au moins une région d'un polypeptide récepteur d'antigènes de cellules T δ , choisie dans le groupe consistant en une région constante, une région variable, 10 une région de jonction et une région de diversité; et l'exposition de la cellule à des conditions telles que les deux séquences d'acides nucléiques sont exprimées par la cellule.
- 15 41. Procédé pour l'expression d'au moins une portion d'un hétérodimère de récepteur d'antigènes de cellules T gamma,delta, comprenant la mise en culture d'une cellule transfectée (a) apte à exprimer un acide nucléique codant pour au moins une région d'un polypeptide récepteur d'antigènes de cellules T gamma choisie dans le groupe consistant en une région variable, une région de jonction et une région constante; et (b) apte à exprimer un acide nucléique codant pour au moins une région d'un polypeptide récepteur d'antigènes de cellules T δ , choisie dans le groupe consistant en une région variable, une région de jonction et une région de diversité; et l'exposition de la cellule à des conditions telles que les deux séquences d'acides nucléiques sont exprimées par la cellule, l'acide nucléique codant pour au moins une région du polypeptide récepteur d'antigènes de cellules T gamma ayant été transfecté dans la cellule.
- 20 42. Procédé selon la revendication 41, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma comprend la Forme 2bc, ayant une masse moléculaire d'environ 40.000 daltons et une séquence comprenant une région constante consistant essentiellement en la séquence d'acides aminés codée par les nucléotides numéros 439 à 1008 de la figure 14, et une région variable.
- 25 43. Procédé selon l'une quelconque des revendications 40, 41 ou 42, dans lequel la cellule est une cellule T.
- 30 44. Procédé selon l'une des revendications 40 ou 41, dans lequel l'acide nucléique codant pour au moins une portion du polypeptide récepteur d'antigènes de cellules T gamma est choisi dans le groupe consistant en (i) un acide nucléique comprenant un unique exon CII; (ii) un acide nucléique comprenant deux exons CII; et (iii) un acide nucléique comprenant trois exons CII.
- 35 45. Procédé selon l'une des revendications 40 ou 41, dans lequel la portion du polypeptide récepteur d'antigènes de cellules T gamma et la portion du polypeptide récepteur d'antigènes de cellules T δ ne sont pas associées par covalence.
- 40 46. Procédé selon l'une des revendications 40 ou 41, dans lequel la portion du polypeptide récepteur d'antigènes de cellules T gamma et la portion du polypeptide récepteur d'antigènes de cellules T δ sont associées par covalence.
- 45 47. Cellules isolées qui expriment au moins une portion d'un hétérodimère de récepteur d'antigènes de cellules T gamma,delta, laquelle portion d'un récepteur d'antigènes de cellules T gamma,delta comprend au moins une région d'un polypeptide récepteur d'antigènes de cellules T gamma choisie dans le groupe consistant en une région constante, une région variable et une région de jonction, associée avec au moins une région d'un polypeptide récepteur d'antigènes de cellules T δ choisie dans le groupe consistant en une région constante, une région variable, une région de jonction et une région de diversité, ledit hétérodimère n'étant pas associé avec un complexe CD3.
- 50 48. Cellules selon la revendication 47, dans lesquelles les cellules sont des cellules épithéliales glandulaires endométriales.
- 55 49. Celle transfectée, qui exprime un hétérodimère de récepteur d'antigènes de cellules T gamma,delta, dans lequel le polypeptide récepteur d'antigènes de cellules T gamma de l'hétérodimère est produit par expression d'un acide nucléique codant pour le polypeptide récepteur d'antigènes de cellules T gamma qui a été transfecté dans la cellule.
50. Cellule selon la revendication 49, dans laquelle le polypeptide récepteur d'antigènes de cellules T gamma



est la Forme 2bc ayant une masse moléculaire d'environ 40.000 daltons et une séquence comprenant une région constante consistant essentiellement en la séquence d'acides aminés codée par les nucléotides numéros 439 à 1008 de la figure 14, et une région variable.

- 5 51. Cellule transfectée, qui exprime au moins une portion d'un hétérodimère récepteur d'antigènes de cellules T gamma,delta, laquelle portion d'un récepteur d'antigènes de cellules T gamma,delta comprend au moins une région d'un polypeptide récepteur d'antigènes de cellules T gamma choisie dans le groupe consistant en une région constante, une région variable et une région de jonction, associée avec au moins une région
- 10 d'un polypeptide récepteur d'antigènes de cellules Tδ choisie dans le groupe consistant en une région constante, une région variable, une région de jonction et une région de diversité; l'hétérodimère n'étant pas associé avec un complexe CD3, la portion du polypeptide récepteur d'antigènes de cellules T gamma étant produite par expression d'un acide nucléique codant pour la portion du récepteur d'antigènes de cellules T gamma qui a été transfecté dans la cellule.
- 15 52. Procédé pour la détermination de l'utilisation relative d'un polypeptide récepteur d'antigènes de cellules T gamma dans un échantillon provenant d'un sujet humain, comprenant:
- (a) la détermination dans un échantillon contenant des cellules T provenant d'un sujet de la quantité d'un polypeptide récepteur d'antigènes de cellules T gamma de Forme 2bc;
- (b) la détermination dans un échantillon provenant d'un sujet de la quantité d'un polypeptide récepteur d'antigènes de cellules T gamma choisi dans le groupe consistant en un polypeptide gamma de Forme 1 et un polypeptide gamma de Forme 2abc; et
- (c) la comparaison de la quantité déterminée dans l'étape (a) avec la quantité déterminée dans l'étape (b), déterminant ainsi l'utilisation relative.
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