Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 2326–2330, April 1989
Immunology

Molecular heterogeneity of γδ T-cell antigen receptors expressed by CD4⁻ CD8⁻ T-cell clones from normal donors: Both disulfide- and non-disulfide-linked receptors are δTCS1⁺

(γ-chain receptors/δ-chain receptors/cytotoxic T cells)

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Contributed by Robert A. Good, December 21, 1988

ABSTRACT **We investigated the molecular heterogeneity of γδ T-cell antigen receptors (TCR) expressed on T-cell clones generated from peripheral blood lymphocytes of normal donors. Extensive molecular heterogeneity was seen at the γ-chain level and, to a lesser extent, at the δ-chain level. Both disulfide and non-disulfide γδ TCR were found and use different γ chains with similar molecular masses (range, 41–43 kDa). In contrast, γ chains of 55–60 kDa, which are expressed on T-cell lines derived from the peripheral blood of patients with immunodeficiency disorders, were not found on T-cell clones derived from the peripheral blood of normal donors. δ chains expressed on these T-cell clones had a molecular mass of 37 kDa and were either disulfide or nondisulfide linked. Significant δ-chain heterogeneity was identified in these clones using the anti-δTCS1 and the anti-TCRδ1 monoclonal antibodies. All clones tested were TCRδ1⁺, whereas only 25% of the clones were δTCS1⁺. The anti-δTCS1 monoclonal antibody stained and immunoprecipitated both disulfide- and non-disulfide-linked γδ TCRs from different T-cell clones from normal donors.**

The second T-cell antigen receptor (TCR) in humans is comprised of two distinct polypeptide chains (γ and δ), and it is expressed on a small (3–10%) subpopulation of peripheral blood lymphocytes and certain thymocytes (1–7). Both disulfide- and non-disulfide-linked γδ TCR have been reported and are associated with the CD3 antigen (1–9). Three types of γ-chain polypeptides have been identified in these receptors. A 55- to 60-kDa non-disulfide-linked γ-chain polypeptide that is expressed on certain T-cell lines derived from patients with immunodeficiency disorders (1, 5) and on PEER (3) or MOLT-17 (10) tumor T-cell lines. A second γ-chain polypeptide is ≈40 kDa and is linked to a δ chain by a disulfide bond; it is expressed on T-cell lines and clones derived from the peripheral blood or thymocytes of normal donors or cerebrospinal fluid (6–9). We and others have previously identified a third CD3-associated γ-chain polypeptide of ≈40 kDa, which is nondisulfide linked and is expressed on T-cell lines derived from the peripheral blood of patients with immunodeficiency disorders (5), normal donors (8, 11), thymocytes (2), and the MOLT-13 T-cell line (11). In this communication we have investigated the molecular heterogeneity of γδ T-cell receptors expressed on T-cell clones derived from peripheral blood lymphocytes from normal donors. Clones with both disulfide- and non-disulfide-linked TCR were identified and all were found to contain γ chains with molecular masses in the range of 40–43 kDa. The δ-chain polypeptides associated with these receptors exhibited a molecular mass of 37 kDa. However, δ-chain heterogeneity was identified in these clones by using two different anti-δ-chain monoclonal antibodies (mAbs).

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MATERIALS AND METHODS

mAbs. Anti-Leu 4, phycoerythrin-conjugated anti-Leu 4 and fluorescein isothiocyanate (FITC)-conjugated anti-WT31 mAbs were purchased from Becton Dickinson. OKT4 and OKT8 mAbs were obtained from Ortho Diagnostics. Anti-βF1 mAb (12), anti-δTCS1 mAb (13), FITC-conjugated anti-δTCS1 mAb, anti-TCRδ1 mAb (14), and FITC-conjugated anti-TCRδ1 mAb were obtained from T Cell Sciences (Cambridge, MA). Cells stained with the anti-δTCS1 mAb were designated in this study δTCS1⁺. Cells stained with the anti-TCRδ1 mAb were designated δ1⁺. A γ-chain-specific mAb, designated 3D5, was developed in this laboratory by immunizing mice with a hybrid protein containing a large part of the CI exon of the human Cγ2 gene segment. This hybrid protein was expressed in *Escherichia coli* using open reading frame vectors (unpublished results). This mAb (IgG1,κ) immunoprecipitated γ chains from denatured lysates of appropriate cells but did not stain live cells. It did not recognize any polypeptide chains in lysates of αβ TCR-expressing T-cell lines and clones.

Preparation of CD3⁺ CD4⁻ CD8⁻ Cells. Human peripheral blood mononuclear cells (PBMC) from normal donors were isolated using Ficoll/Hypaque density cushions. Nonadherent lymphocytes were isolated using a nylon/wool column (15) and were primarily T lymphocytes (≈95% CD3⁺). Nonadherent lymphocytes were treated with anti-CD4 (OKT4) and anti-CD8 (OKT8) mAb plus rabbit complement, as described (16). Double-negative cells were isolated on a Ficoll/Hypaque density cushion and were found to contain 40–60% CD3⁺ cells, ≈5% WT31⁺ cells, and 3% CD4⁺ or CD8⁺ cells.

Generation of T-Cell Clones. Viable double-negative PBMC were stimulated under limiting-dilution conditions (1, 3, 10, or 30 cells per well) with a mixture of allogeneic PBMC (100,000 cells per well, irradiated with 5000 rads; 1 rad = 0.01 Gy) from two or three normal donors (feeder cells), recombinant interleukin 2 (rIL-2; 100 units/ml) and phytohemagglutinin (10 μg/ml). Fresh feeder cells were added to the cultures every 10 days. Clones derived from these cultures were expanded for analysis. T-cell clones were developed in RPMI 1640 medium supplemented with 10% fetal calf serum,

Abbreviations: TCR, T-cell antigen receptors; mAb, monoclonal antibody; PBMC, peripheral blood mononuclear cells; δ1, TCR-δ1; FITC, fluorescein isothiocyanate; V, J, and C regions, variable, joining, and constant regions.

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25 mM Hepes buffer, 2.5 mM L-glutamine, penicillin at 100 units/ml, and streptomycin at 100 μg/ml (all purchased from GIBCO). In certain experiments AIM V medium (GIBCO) was used instead of RPMI 1640 medium.

Immunofluorescence Staining. Cell-surface immunofluorescence staining was done as described (17). Immunofluorescence analysis was done using an Epics profile analyzer (Coulter).

Cell-Mediated Cytotoxicity. Cell-mediated cytotoxicity was determined using a ^{51}Cr release assay as described (16, 17).

Immunoprecipitation. Cells were labeled with $Na^{125}I$ (specific activity, 14.8 mCi/μg of iodine; 1 Ci = 37 GBq; Amersham) by using the lactoperoxidase method and lysed in buffer consisting of 0.01 M Tris·HCl (pH 8.0), 0.15 M NaCl, 1 mM phenylmethylsulfonyl fluoride, aprotinin at 20 kallikrein-inhibiting units/ml, ovomucoid trypsin inhibitor at 2 mg/ml, 0.01% NaN_3, supplemented with either 5 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) or 1% Nonidet P-40 (all purchased from Sigma). Lysates were precleared by three subsequent incubations with 10 mg of PANSORBIN (Calbiochem–Behring) coated with normal mouse IgG. Specific immunoprecipitation was carried out with PANSORBIN coated with 2–5 μg of specific mAbs. For immunoprecipitations with the anti-γ-chain-specific 3D5 mAb, lysates were denatured and alkylated as described (5). Samples were analyzed by SDS/PAGE by use of 10% or 12.5% acrylamide gels. Gels were dried and visualized by autoradiography at −70°C on Kodak XAR-5 film using intensifying screens.

Northern (RNA) Blotting. Northern blotting was done by standard methods (18, 19). The human α-chain-specific pY1.4 (18) and the human β-chain-specific Jur-β2 (19) cDNA probes were provided by T. Mak (Ontario Cancer Institute, University of Toronto, Toronto). The human γ-chain specific pTγ1 probe (20) was provided by T. Quertermous and J. Seidman (Harvard Medical School, Boston).

RESULTS

Several different $CD3^+$ $WT31^-$ $CD4^-$ $CD8^-$ T-cell clones were generated from six different donors, as described. These were analyzed by cell-surface immunofluorescence, and their functional properties were investigated. Four out of sixteen clones were $CD3^+$ $WT31^-$ $\delta TCS1^+$, whereas the remaining twelve were $CD3^+$ $WT31^-$ $\delta TCS1^-$ (Table 1). Eight of these $\delta TCS1^-$ clones were tested with the anti-TCRδ1 mAb, and all were found positive ($\delta TCS1^-$ $\delta1^+$). In addition, six clones of the $CD3^+$ $WT31^+$ $CD4^-$ $CD8^-$ $\delta TCS1^-$ $\delta1^-$ phenotype were obtained from the same donors. Representative results of the immunofluorescence staining profiles are shown in Fig. 1. These $CD3^+$ $WT31^-$ T-cell clones exhibited variable levels of nonspecific cytotoxicity and lysed cells of the K-562 tumor cell line, cells of the MEL-21 melanoma tumor cell line, OKT3 mAb-producing murine hybridomas (Table 2) and PBMC-stimulated with phytohemagglutinin either unrelated or autologous to the stimulating cells (data not shown).

The γδ TCR of representative clones were analyzed biochemically. The G3.2 clone ($CD3^+$ $WT31^-$ $CD4^-$ $CD8^-$ $\delta TCS1^+$ $\delta1^+$) expressed CD3-associated disulfide-linked γδ TCR. Immunoprecipitation and SDS/PAGE under nonreducing conditions with either the anti-Leu 4 mAb or the anti-δTCS1 mAb or the anti-γ 3D5 mAb revealed a band of 80 kDa. SDS/PAGE under reducing conditions revealed two bands of 38 kDa and 41 kDa (Fig. 2).

Biochemical analysis of the J1.2 clone ($CD3^+$ $WT31^-$ $CD4^-$ $CD8^-$ $\delta TCS1^+$ $\delta1^+$) revealed a non-disulfide-linked TCR. Immunoprecipitation and SDS/PAGE analysis under nonreducing conditions with the anti-Leu 4 mAb revealed (in addition to the CD3 bands) two polypeptide chains of 42 kDa and 37 kDa (Fig. 3). SDS/PAGE analysis under nonreducing conditions revealed that the 42-kDa band was also immunoprecipitated from denatured nonreduced lysates by the 3D5 anti-γ mAb, demonstrating that this band is a γ-chain polypeptide. Immunoprecipitation by the anti-Leu 4 mAb followed by SDS/PAGE analysis under reducing conditions revealed a broad band of 40–42 kDa. The 37-kDa band was not detected under reducing conditions. This finding provides additional evidence suggesting that this band is the δ chain. It is well established (8, 11, 21) that the apparent electrophoretic mobility under reducing conditions in SDS/PAGE of the δ-chain polypeptide is decreased to a relative molecular mass of ≈40 kDa. Therefore, under reducing conditions the δ-chain band overlaps with the γ-chain band. SDS/PAGE analysis under reducing conditions revealed that a polypeptide chain of ≈42 kDa was immunoprecipitated by the anti-γ 3D5 mAb from denatured nonreduced lysates of the J1.2 cells (Fig. 3).

Biochemical analysis of a $CD3^+$ $WT31^-$ $CD4^-$ $CD8^-$ $\delta TCS1^-$ $\delta1^+$ T-cell clone designated A8.2.20 revealed the

Table 1. Surface phenotype of $CD3^+$ $CD4^-$ $CD8^-$ T-cell clones from normal donors

Clone	Phenotype					
	α/β TCR(WT31)	δTCS1	TCRδ1	Leu-19	Leu-7	CD16
A8.1.11	−	−		+	−	−
A8.2.18	−	−				
A8.2.20	−	−	+	+	−	−
A8.39	−	−	+	+	−	−
A9.3	−	−		+	−	−
A9.5	−	+		+		
A9.15	−	−		+		
G3.2	−	+	+	−	−	−
G3.13	−	+		−	−	
G8.5	−	−		−	−	−
J1.2	−	+	+	−		
J1.4	−	−		−		
S2.1	−	−	+	−	−	−
S2.3	−	−	+	−	−	−
S2.4	−	−	+	−	−	−
S2.14	−	−	+			
S2.5	+	−	−			
S2.9	+	−	−			
A8.1.1	+	−	−	−	−	−
A8.30	+	−	−	−	−	−
G3.9	+	−				
G3.15	+	−	−	−	−	−

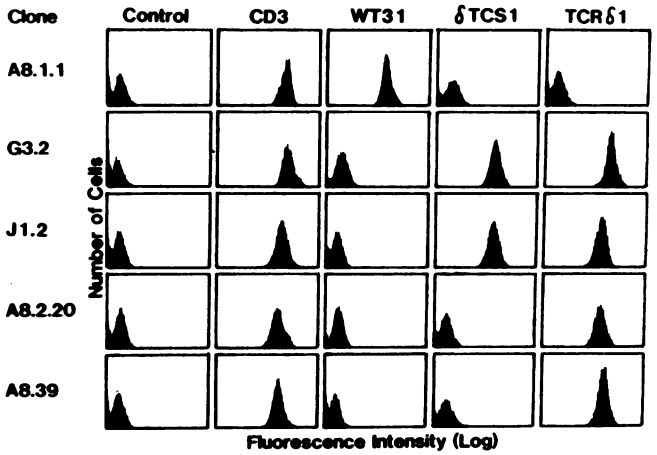

FIG. 1. Immunofluorescence analysis of $CD3^+$ $CD4^-$ $CD8^-$ T-cell clones. The cells were stained with phycoerythrin-conjugated anti-Leu 4, FITC-conjugated WT31, FITC-conjugated anti-δTCS1, or FITC-conjugated anti-TCRδ1. Control cells were stained with FITC-conjugated goat anti-mouse IgG antibody.

Table 2. Cytolytic activity of CD3$^+$ CD4$^-$ CD8$^-$ T-cell clones

Clone	Phenotype WT31	Phenotype δTCS1	% ^{51}Cr release (E/T = 20:1) K-562 Medium	K-562 Anti-CD3*	MEL21	OKT3-Hyb†	OKT4-Hyb†
A8.1.11	−	−	14	34	24	50	ND
A8.2.18	−	−	5	46	ND	61	ND
A8.2.20	−	−	4	47	2	58	1
A8.39	−	−	14	16	9	53	1
A9.3	−	−	32	27	17	66	0
G8.5	−	−	2	52	36	77	1
S2.1	−	−	0	43	1	ND	ND
S2.4	−	−	34	68	64	ND	ND
A9.5	−	+	5	53	2	71	5
G3.2	−	+	5	3	6	28	0
J1.2	−	+	1	1	ND	ND	ND
A8.1.1	+	−	0	0	0	6	0
A8.30	+	−	0	0	2	10	0
G3.9	+	−	0	0	0	18	0
G3.15	+	−	12	54	2	40	0

ND, not done. E/T, effector cell/target cell ratio.
*Effector cells were incubated for 30 min with 1:100 dilution of ascites of anti-CD3 mAb.
†Anti-CD3 or anti-CD4 mAb-secreting murine hybridomas.

presence of a disulfide-linked TCR. Under nonreducing conditions a band of ≈80 kDa was immunoprecipitated by the anti-Leu 4 mAb or the anti-γ 3D5 mAb. SDS/PAGE analysis under reducing conditions of the material immunoprecipitated by the anti-Leu 4 mAb or the anti-γ 3D5 mAb revealed two polypeptide chains of 43 kDa and 37 kDa (Fig. 4). The 37-kDa band was clearly visible in the immunoprecipitations with both antibodies; however, it was more pronounced in the immunoprecipitation by the anti-Leu 4 mAb than by the anti-γ 3D5 mAb. Immunoprecipitation with the anti-γ 3D5 mAb was carried out from denatured nonreduced lysates. Immunoprecipitation with the anti-TCRδ1 mAb followed by SDS/PAGE analysis under reducing conditions revealed two polypeptide chains of 37 kDa and 43 kDa (Fig. 4). Because the γδ TCR of the A8.2.20 clone is disulfide linked, it cannot be determined whether the lower-molecular mass chain (presumably the δ chain) exhibits a decrease in apparent electrophoretic mobility under reducing conditions comparable to that seen with the other clones. Biochemical analysis of the TCR molecules from the A8.1.1 T-cell clone (CD3$^+$ WT31$^+$ CD4$^-$ CD8$^-$ δTCS1$^-$ δ1$^-$), which expressed the αβ TCR, is shown for the purpose of comparison (Fig. 5). Immunoprecipitation with the anti-Leu 4 mAb or the anti-βF1 mAb followed by SDS/PAGE analysis under nonreducing conditions revealed a band of 87 kDa (Fig. 5). SDS/PAGE analysis under reducing conditions revealed a broad band of 44–47 kDa. The anti-γ-chain-specific 3D5 mAb did not immunoprecipitate any bands from cells of the A8.1.1 clone or from αβ TCR expressing T-cell lines.

Northern blotting analysis revealed that the A8.2.20 (CD3$^+$ WT31$^-$ δTCS1$^-$ δ1$^+$) T-cell clone expressed full-length γ-chain mRNA (Fig. 6). In contrast, it expressed only low levels of truncated α- and β-chain mRNAs. The G3.2 (CD3$^+$ WT31$^-$ δTCS1$^+$ δ1$^+$) T-cell clone expressed full-length γ-chain transcript and low levels of truncated α- and β-chain mRNA. The A8.1.1 CD3$^+$ WT31$^+$ δTCS1$^-$ T-cell clone expressed full-length α- and β-chain transcripts but not γ-chain mRNA.

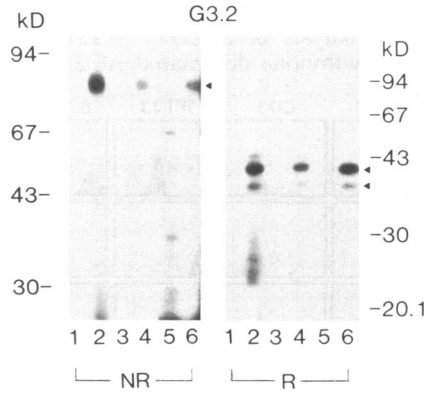

FIG. 2. Cells of the G3.2 T-cell clone (CD3$^+$ WT31$^-$ CD4$^-$ CD8$^-$ δTCS1$^+$ δ1$^+$) were labeled with ^{125}I and lysed in 5 mM CHAPS lysis buffer (lanes 1 and 2) or 1% Nonidet P-40 lysis buffer (lanes 3–6). Lysates were immunoprecipitated under nonreducing conditions with mouse IgG (lanes 1, 3, and 5), anti-Leu 4 mAb (lane 2), anti-δTCS1 mAb (lane 4), or anti-γ chain mAb 3D5 (lane 6). Immunoprecipitations with the 3D5 mAb were carried out from denatured nonreduced lysates as described. Samples were solubilized in SDS sample buffer with β-mercaptoethanol (reducing conditions; R) or without (nonreducing conditions; NR) and subjected to electrophoresis on 10% (NR) or 12.5% (R) SDS/PAGE gels.

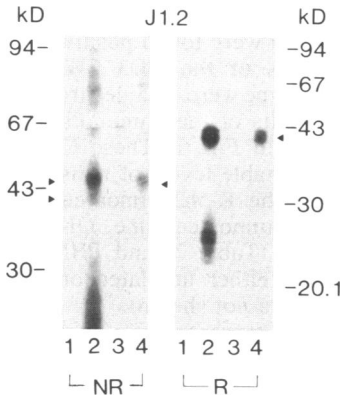

FIG. 3. Cells of the J1.2 T-cell clone (CD3$^+$ WT31$^-$ CD4$^-$ CD8$^-$ δTCS1$^+$ δ1$^+$) were labeled with ^{125}I and lysed in 5 mM CHAPS lysis buffer (lanes 1, 2) or 1% Nonidet P-40 lysis buffer (lanes 3 and 4). Lysates were immunoprecipitated with mouse IgG (lanes 1 and 3), anti-Leu 4 mAb (lane 2), or anti-γ chain mAb 3D5 (lane 4). Immunoprecipitations with the 3D5 mAb were carried out from denatured nonreduced lysates as described. Samples were solubilized in SDS sample buffer with β-mercaptoethanol (reducing conditions; R) or without (nonreducing conditions; NR), and subjected to electrophoresis on 10% (NR) or 12.5% (R) SDS/PAGE gels.

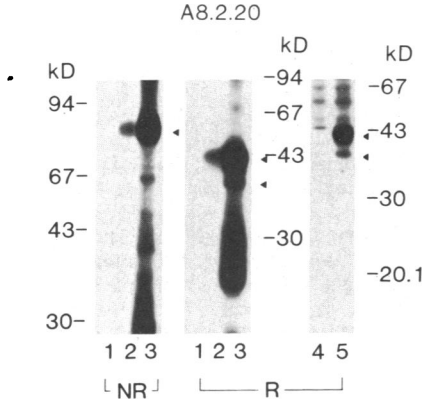

FIG. 4. Cells of the A8.2.20 T-cell clone ($CD3^+$ $WT31^-$ $CD4^-$ $CD8^-$ $\delta TCS1^-$ $\delta 1^+$) were labeled with ^{125}I and lysed in 5 mM CHAPS lysis buffer (lane 3) or 1% Nonidet P-40 lysis buffer (lanes 1, 2, 4, and 5). Lysates were immunoprecipitated with mouse IgG (lanes 1 and 4), anti-γ chain mAb 3D5 (lane 2), anti-Leu 4 mAb (lane 3), or anti-TCRδ1 mAb (lane 5). Immunoprecipitations with the 3D5 mAb were carried out from denatured nonreduced lysates as described. Samples were solubilized in SDS sample buffer with β-mercaptoethanol (reducing conditions; R) or without (nonreducing conditions; NR), and subjected to electrophoresis on 10% (NR) or 12.5% (R) SDS/PAGE gels.

DISCUSSION

These results demonstrate significant molecular heterogeneity of the $\gamma\delta$ TCR. Both disulfide and non-disulfide-linked $\gamma\delta$ TCR were expressed on T-cell clones derived from the peripheral blood of normal donors. Extensive heterogeneity of the γ chain and, to a lesser degree, of the δ chain was observed. Disulfide-linked and non-disulfide-linked $\gamma\delta$ TCR expressed on T-cell clones derived from the peripheral blood of normal donors employed different γ chains, although the TCR had similar molecular masses (41–43 kDa). These γ-chain polypeptides are coded by different γ-gene segments. The disulfide-linked 41- to 43-kDa γ-chain polypeptide is encoded by the constant (C) γ1 gene segment, whereas the

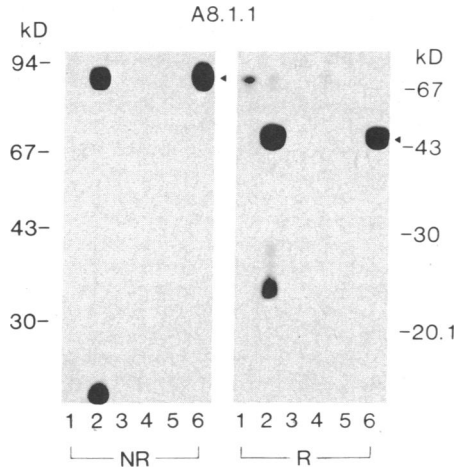

FIG. 5. Cells of the A8.1.1 T-cell clone ($CD3^+$ $WT31^+$ $CD4^-$ $CD8^-$ $\delta TCS1^-$) were labeled with ^{125}I and lysed in 5 mM CHAPS lysis buffer (lanes 1 and 2) or 1% Nonidet P-40 lysis buffer (lanes 3–6). Lysates were immunoprecipitated with mouse IgG (lanes 1, 3, and 5), anti-Leu 4 mAb (lane 2), anti-γ chain mAb 3D5 (lane 4), or anti-βF1 mAb (lane 6). Immunoprecipitations with the 3D5 mAb were carried out from denatured nonreduced lysates as described. Samples were solubilized in SDS sample buffer with β-mercaptoethanol (reducing conditions; R) or without (nonreducing conditions; NR) and subjected to electrophoresis on 10% (NR) or 12.5% (R) SDS/PAGE gels.

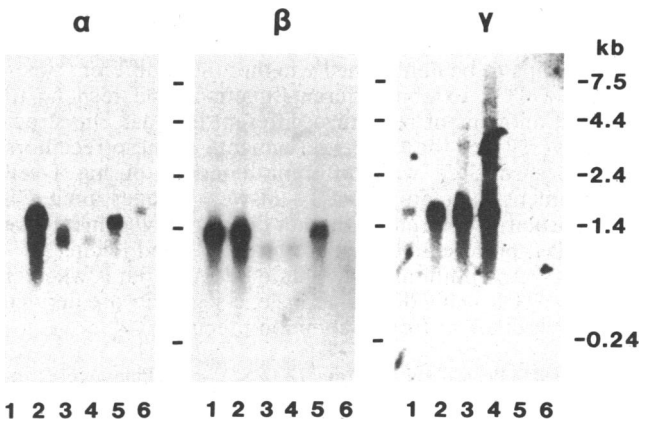

FIG. 6. Blot hybridization analysis of RNA isolated from various T-cell clones and other lines. α-, β-, and γ-chain-specific cDNA probes are described elsewhere. The samples are as follows: 1, PEER; 2, HPB-ALL; 3, G3.2; 4, A8.2.20; 5, A8.1.1; 6, Epstein–Barr virus-transformed B cell.

non-disulfide-linked 41- to 43-kDa γ-chain polypeptide is encoded by a Cγ2 gene segment containing two CII exons (11, 21, 22). The γ-chain polypeptide of 55–60 kDa that we and others (1, 3, 5, 10) have found on T-cell lines derived from the peripheral blood of patients with primary immunodeficiency disorders or on certain tumor T-cell lines is encoded by a Cγ2 gene segment that contains three CII exons instead of two (11, 21, 22). This γ-chain polypeptide of 55–60 kDa was not found in any of the T-cell clones or lines derived from the peripheral blood of normal donors, although this polypeptide appears to be the most frequently observed γ chain on T-cell lines derived from the peripheral blood of patients with primary immunodeficiency disorders (1, 5). Whether or not $\gamma\delta$ TCR using different γ-chain polypeptides exhibit different functions remains to be investigated; the fact that they are expressed on cells of different differentiation or maturation stages supports this possibility. Although the 55- to 60-kDa γ-chain polypeptide has not been found on T-cell clones or lines derived from the peripheral blood from normal donors, we have recently developed from tumor-infiltrating lymphocytes from a patient with Wilms tumor a T-cell line that expressed a non-disulfide-linked $\gamma\delta$ TCR, using a 60-kDa γ-chain polypeptide (data not shown).

All δ chains, identified in these T-cell clones from the peripheral blood of normal donors, as well as those on other T-cell clones generated from tumor-infiltrating lymphocytes from patients with metastatic melanoma or Wilms tumor (unpublished results), exhibited essentially the same molecular mass (37–40 kDa) (Figs. 2 and 3 and unpublished results), suggesting that only one δ-chain gene segment is used. This was the case in both disulfide- and non-disulfide-linked $\gamma\delta$ TCR. The small differences in molecular mass (range 37- to 40-kDa) are probably due to the use of different variable (V) δ chains. However, significant δ-chain heterogeneity was identified using the anti-δTCS1 (13) and the anti-TCRδ1 (14) mAbs. All $\gamma\delta$ clones examined, generated from the peripheral blood of normal donors or from tumor-infiltrating lymphocytes (unpublished results) were $\delta 1^+$, whereas $\approx$25% of these clones were $\delta TCS1^+$. Several clones were $\delta TCS1^-$ $\delta 1^+$. Because the anti-δTCS1 mAb recognizes a Vδ determinant (13), this heterogeneity may be due to the use of different variable regions of the δ chain. Furthermore, the anti-δTCS1 mAb stained and immunoprecipitated both disulfide-linked and non-disulfide-linked $\gamma\delta$ TCR expressed on T-cell clones derived from either peripheral blood from normal donors or tumor-infiltrating lymphocytes from patients with metastatic melanoma (unpublished results). These findings contrast

with those of Bottino *et al.* (23), who reported that δTCS1$^+$ cells express only non-disulfide-linked receptors.

It remains to be determined whether these different types of the $\gamma\delta$ TCR exhibit different functions and respond to different antigens or recognize different ligands. The structural diversity of the Cγ gene segments in these receptors may be associated with different functions during T-cell development and maturation. To answer this question it will be important to determine whether different Cγ segments are rearranged preferentially with different Vγ and joining (J) γ segments. An additional question to be answered is whether or not $\gamma\delta$ TCR using different Cγ gene segments are derived from each other or from a common precursor.

Note Added in Proof. Four additional δTCS$^+$ T-cell clones expressing disulfide-linked $\gamma\delta$ TCR have been recently developed in our laboratory. One of these clones was derived from peripheral blood T lymphocytes from a normal donor, two clones were from a patient with partial DiGeorge syndrome, and one clone was from tumor-infiltrating lymphocytes from a patient with malignant melanoma.

This work was supported in part by Grants AI-24669 and AG-05628 from the National Institutes of Health, Grant CH-420 from the American Cancer Society, a grant from the Eleanor Naylor Dana Charitable Trust and a grant from The University Cancer Foundation of the M. D. Anderson Cancer Center.

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