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Research Article

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Disulfide-linked γδ T Cell Antigen Receptors Expressed on T Cells Derived from Patients with Primary Immunodeficiency Disorders

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Abstract

T cell lines or clones from two patients, one with a partial DiGeorge syndrome and one with severe common variable immunodeficiency expressed disulfide-linked γδ T cell antigen receptor (TCR) comprised of a γ-chain polypeptide of 40–43 kD, and a δ-chain polypeptide of 37–40 kD. This γδ TCR appears to be similar to that found on T cell clones, and lines derived from peripheral blood lymphocytes from normal donors. Previous studies have shown that T cell lines derived from the peripheral blood of patients with immunodeficiency disorders express non–disulfide-linked γδ TCR. In contrast to the latter and coincident with findings in the present study, the vast majority of T cell lines and clones derived from the peripheral blood of normal donors express disulfide-linked γδ TCR.

Introduction

The γδ T cell antigen receptor (TCR)[1] is comprised of a γ- and a δ-chain polypeptide associated either noncovalently (non–disulfide-linked TCR) or by a disulfide bond (disulfide-linked TCR) (1). Although only one δ-chain constant region gene segment has been identified (2–4), three different forms of the γ-chain have been demonstrated (1, 5–12). These are: (*a*) A disulfide-linked γ-chain polypeptide of 40 kD, coded by the Cγ1 gene segment, which is expressed on T cells derived from the peripheral blood or thymocytes from normal donors (1, 9, 10). (*b*) A non–disulfide-linked γ-chain polypeptide of 40 kD, coded by a Cγ2 gene segment containing two copies of the CII exon and expressed on T cells derived from the peripheral blood of patients with primary immunodeficiency disorders (8), certain normal donors (1, 11, 12) and on a thymocyte clone (6). (*c*) A non–disulfide-linked γ-chain polypeptide of 55–60 kD, coded by a Cγ2 gene segment containing three copies of the CII exon, and expressed primarily on T cells derived from patients with immunodeficiency disorders (5, 8), and the Peer (13) and Molt-17 (14) tumor cell lines. Our previous studies (8) and studies of others (1, 5) have shown that T cell lines derived from the peripheral blood of patients with primary immunodeficiency disorders expressed non–disulfide-linked γδ TCRs. In contrast, the vast majority of T cell clones or lines derived from the peripheral blood of normal donors expressed disulfide-linked γδ TCR. We report here that T cell lines and clones derived from the peripheral blood from two patients with partial DiGeorge syndrome or a severe form of common variable immunodeficiency expressed disulfide-linked γδ TCR.

Methods

Patients. Peripheral blood lymphocytes from a patient with partial DiGeorge syndrome and a patient with severe common variable immunodeficiency (CVI) were used in this study. The patient with partial DiGeorge syndrome had characteristic dismorphic facial features, no thymic shadow, and only very low levels of thymulin (15). The patient with severe CVI had significant T cell immunologic abnormalities, besides antibody deficiency.

Monoclonal antibodies. OKT4 and OKT8 MAb were obtained from Ortho Diagnostic Systems Inc. (Raritan, NJ). Anti-δTCS1 MAb, anti-TCRδ1 MAb, FITC-conjugated anti-δTCS1 MAb, and FITC-conjugated anti-TCRδ1 MAb were purchased from T Cell Sciences (Cambridge, MA). Cells stained with the anti-δTCS1 MAb or with the anti-TCRδ1 MAb were designated in this study as $\delta TCS1^+$ or $\delta 1^+$, respectively. Anti-Leu 4, phycoerythrin-conjugated anti-Leu 4, and FITC-conjugated anti-WT31 MAbs were purchased from Becton-Dickinson (Sunnyvale, CA). An anti-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 (16). This MAb immunoprecipitates all three forms of γ-chain polypeptides from denatured lysates. It recognizes both the Cγ1 and Cγ2 gene segments. It is γ-chain specific and does not immunoprecipitate αβ TCR (16).

T cell lines and clones. Nonadherent peripheral blood lymphocytes from patients with immunodeficiency, or from normal donors were prepared as previously described (17). The cells were treated with OKT4 and OKT8 MAbs, plus rabbit complement as described (17). $CD4^-CD8^-$ lymphocytes were isolated by centrifugation on a Ficoll-Hypaque density cushion and were found to contain 40–60% $CD3^+$ cells, ~ 5% $WT31^+$ cells, and 3% $CD4^+$ or $CD8^+$ cells. To generate T cell lines, the cells were cultured at a concentration of 1×10^6/ml in 24-well tissue culture plates in RPMI 1640 supplemented with 10% fetal calf serum, 25 mM Hepes buffer, 2 mM L-glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin (all from Gibco Laboratories, Grand Island, NY), 100 U/ml of highly purified recombinant human interleukin-2 (rIL-2) (Cetus Corp., Emeryville, CA), 5 μg/ml of PHA, and irradiated (5,000 rad) allogeneic peripheral blood mononuclear cells as feeders (2×10^6/well). The cultures were fed every 4 d. T cell lines were harvested 4–5 wk later. To generate T cell clones, purified $CD4^-CD8^-$ peripheral blood lymphocytes were stimulated under lim-

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1. *Abbreviations used in this paper:* CVI, common variable immunodeficiency; TCR, T cell antigen receptor.

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iting dilution conditions (1, 3, 10, or 30 cells per well) with irradiated (5,000 rad) allogeneic mononuclear leukocytes from a normal donor (100,000 cells per well), rIL-2 (100 U/ml), and PHA (5 μg/ml), as previously described (12). Clones derived from these cultures were expanded for analysis.

Immunofluorescence analysis. Cell surface immunofluorescence was carried out as previously described (18).

Cell-mediated cytotoxicity. Cell-mediated cytotoxicity was determined by a ^{51}Cr-release assay as described (18).

Immunoprecipitation. Cells were labeled with Na^{125}I (14.8 mCi/μg sp act of iodine) by the lactoperoxidase method and immunoprecipitations were carried out as previously described (12). For immunoprecipitations with the anti-γ chain specific 3D5 MAb, lysates were denatured and alkylated as described previously (12, 16). Samples were analyzed by SDS/PAGE, either under reducing or nonreducing conditions.

Results

Two CD4$^-$CD8$^-$ T cell lines exhibiting substantial proportions of cells expressing γδ TCR were developed by expanding in culture purified CD4$^-$CD8$^-$ peripheral blood lymphocytes from a patient with severe CVI and a patient with partial DiGeorge syndrome. Immunofluorescence analysis of these lines is shown in Fig. 1. The CVI cell line was 99% CD3$^+$, 86% δ1$^+$, 25% δTCS1$^+$, and 11% WT31$^+$. The DiGeorge line was 99% CD3$^+$, 51% δ1$^+$, 43% δTCS1$^+$, and 39% WT31$^+$ (Fig. 1). Two T cell clones were derived from CD4$^-$CD8$^-$ cells from the patient with partial DiGeorge Syndrome, and both were CD3$^+$CD4$^-$CD8$^-$WT31$^-$δTCS1$^+$δ1$^+$. These T cell lines and clones exhibited MHC unrestricted cytotoxicity and lysed cells of the K562 cell line and of the MEL21 melanoma tumor cell line (Table I). Cytotoxicity by the DiGeorge T cell clones against the K562 targets was enhanced by the OKT3 MAb.

Immunoprecipitation of ^{125}I-labeled lysates of the DiGeorge T cell line by anti-Leu 4 MAb, followed by SDS/PAGE analysis under nonreducing conditions revealed polypeptide bands in the range of 83–93 kD (in addition to the CD3 bands of 20–28 kD). SDS/PAGE analysis under reducing conditions demonstrated two polypeptide chains with molecular masses of 40 and 36 kD, in addition to the CD3 bands (Fig. 2).

Immunoprecipitation, by the γ-chain specific 3D5 MAb, of nonreduced denatured lysates from two T cell clones (CD3$^+$WT31$^-$CD4$^-$CD8$^-$δTCS1$^+$δ1$^+$) derived from CD4$^-$CD8$^-$ peripheral blood lymphocytes from the patient with partial DiGeorge syndrome, demonstrated that they also expressed disulfide-linked γδ TCR. SDS-PAGE analysis under nonreducing conditions revealed on both clones polypeptides of 81 kD, which were resolved to two polypeptide bands of 37 and 40 kD under reducing conditions (Fig. 2). These two T cell clones were δTCS1$^+$, demonstrating that both disulfide- and non–disulfide-linked γδ TCR can express the δTCS1 determinant (12).

Biochemical analysis of the T cell line CD3$^+$WT31$^-$CD4$^-$CD8$^-$δ1$^+$ derived from the patient with severe CVI demonstrated that it also expressed a disulfide-linked γδ TCR. Im-

Table I. Cytolytic Activity of γδ$^+$TCR T Cell Lines or Clones from Patients with Immunodeficiency Diseases

	% ^{51}Cr release (E/T = 20:1)			
	K562		MEL21	
Line or clone	Medium	Anti-CD3	Medium	Anti-CD3
CVI T cell line	79.4±5.2	86.5±7.9	50.7±2.4	25.4±2.5
DiGeorge T cell line	53.7±2.1	ND	19.7±3.0	ND
DiGeorge T cell clone	28.7±2.1	85.6±1.6	2.1±0.3	6.1±6.0
DiGeorge T cell clone	15.6±4.1	63.6±4.1	0.1±1.2	4.8±1.6

ND, not done.

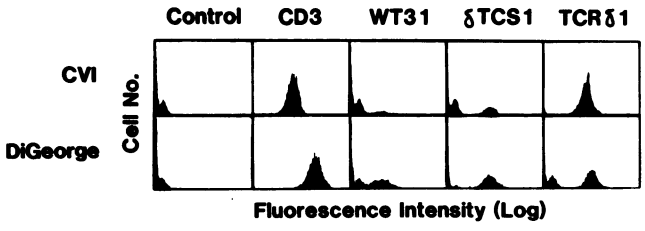

Figure 1. Immunofluorescence analysis of T cell lines derived from CD4$^-$ CD8$^-$ peripheral blood lymphocytes from a patient with severe CVI and a patient with partial DiGeorge syndrome. The cells were stained with FITC-conjugated WT31; FITC-conjugated anti-δTCS1; FITC-conjugated anti-TCRδ1, or phycoerythrin-conjugated anti-Leu 4. Control cells were stained with FITC-conjugated goat anti-mouse IgG antibody.

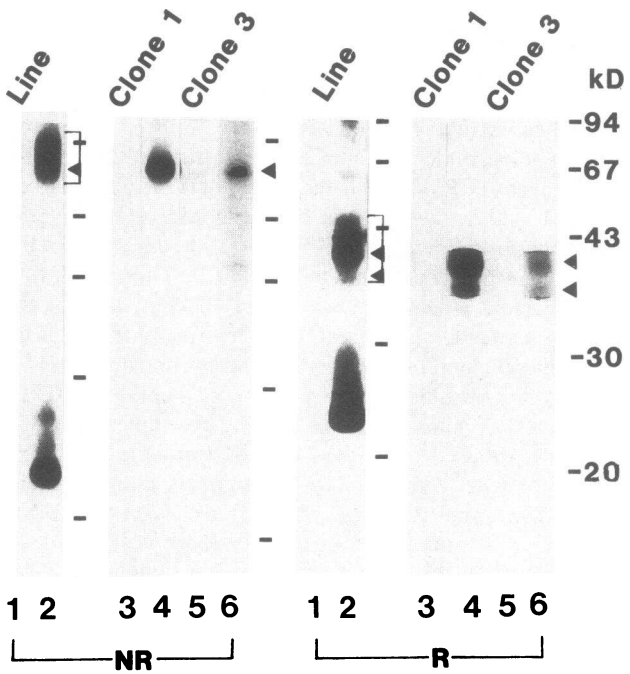

Figure 2. Cells from a T cell line and two T cell clones (CD3$^+$WT31$^-$CD4$^-$CD8$^-$δTCS1$^+$δ1$^+$) derived from peripheral blood lymphocytes from a patient with partial DiGeorge syndrome were labeled with ^{125}I and lysed in 5 mM 3[(3-cholamidopropyl)dimethyl-ammonio]1-propanesulfonate lysis buffer (lanes *1* and *2*), or 1% NP-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*), or anti-γ chain 3D5 MAb (lanes *4,* and *6*). Immunoprecipitations with the anti-γ chain 3D5 MAb were carried out after denaturation of the lysates as described in Methods. Samples were solubilized in SDS sample buffer with β-mercaptoethanol (reducing conditions, *R*) or without (nonreducing conditions, *NR*), and subjected to electrophoresis on 12.5% SDS-PAGE gels.

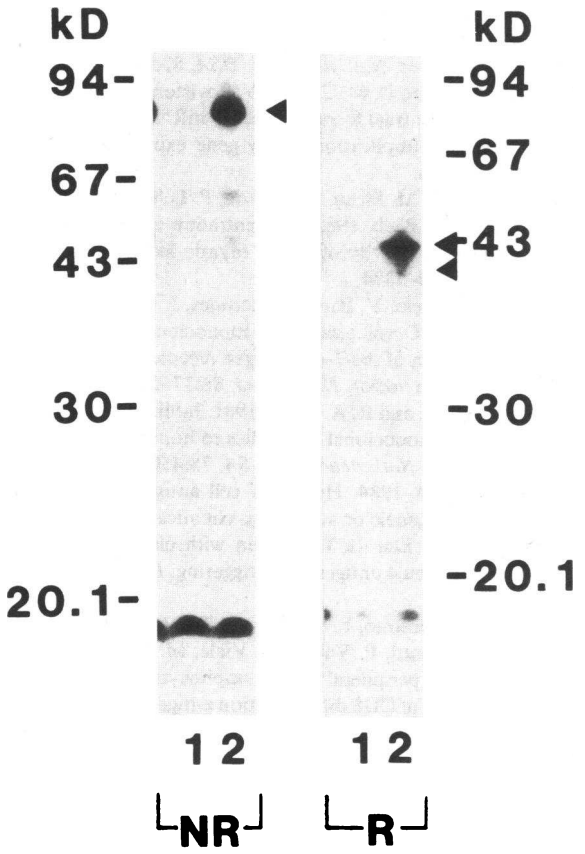

Figure 3. Cells from a T cell line derived from peripheral blood lymphocytes from a patient with severe CVI were labeled with ^{125}I and lysed in 1% NP-40 lysis buffer. Lysates were immunoprecipitated under nonreducing conditions with mouse IgG (lane *1*), or anti-γ chain 3D5 MAb (lane *2*). Immunoprecipitations with the anti-γ 3D5 MAb were carried out after denaturation of the lysates as described in Methods. Samples were solubilized in SDS sample buffer with β-mercaptoethanol (reducing conditions, *R*) or without (nonreducing conditions, *NR*), and subjected to electrophoresis on 12.5% SDS-PAGE gels.

munoprecipitation of denatured nonreduced lysates by the anti-γ 3D5 MAb followed by SDS-PAGE analysis under nonreducing conditions revealed a band of 81 kD. Under reducing conditions, two bands of 40 and 43 kD were observed (Fig. 3).

Discussion

T cell lines and clones derived from peripheral blood lymphocytes from a patient with partial DiGeorge syndrome and a patient with severe CVI expressed disulfide-linked $\gamma\delta$ TCR. These $\gamma\delta$ TCR appeared to use a γ-chain polypeptide of $\sim$ 40–43 kD and a δ-chain of 37–40 kD and appeared to be similar to the $\gamma\delta$ TCR found on T cell lines or clones derived from normal donors. T cell lines derived from the peripheral blood of three patients with primary immunodeficiency disorders expressed exclusively non-disulfide linked $\gamma\delta$ TCR using either a 40-kD or a 55–60-kD γ-chain polypeptide (8). Similarly, Brenner et al. (1, 5) observed only non-disulfide-linked γ-chain polypeptides on T cell lines derived from the peripheral blood of certain patients with primary immunodeficiency disorders. Our results suggest that T cell lines derived from peripheral blood lymphocytes from patients with primary immunodeficiency disorders can also express disulfide-linked $\gamma\delta$ TCR.

Our findings (12, and unpublished observations), and those of others (19) suggest that $\sim$ 70–80% of the $\gamma\delta$ TCR T cell clones or lines derived from peripheral blood lymphocytes from normal donors expressed disulfide-linked $\gamma\delta$ TCR. The remaining clones expressed non-disulfide linked $\gamma\delta$ TCR, using the 40 kD Cγ2 γ-chain polypeptide (11, 12, 19). $\gamma\delta$ TCR expressing T cells derived from normal donors, and using the 55–60-kD non-disulfide-linked γ-chain polypeptide have been rare. Moretta et al. (19) have reported the utilization of the 55–60-kD γ-chain by 2% of the $\gamma\delta$ TCR T cell clones from normal donors that they examined. Also, they found a correlation of the expression of the CD8 T cell differentiation antigen with the 55–60-kD non-disulfide-linked γ-chain polypeptide. We have also obtained one $\gamma\delta$ TCR expressing T cell clone derived from peripheral blood lymphocytes of a normal donor that used the 55–60-kD non-disulfide-linked γ-chain polypeptide. These cells were $CD8^-$ (manuscript in preparation).

The T cell line and the T cell clones from the patient with the partial DiGeorge syndrome expressed the δTCS1 determinant. Similarly, $\sim$ 25% of the $\gamma\delta$ TCR-expressing cells of the T cell line from the patient with severe CVI were $\delta TCS1^+$. All these T cell lines and clones expressed disulfide-linked $\gamma\delta$ TCR in agreement with our previous findings (12) and in contrast to those of Bottino et al. (20), who reported that the δTCS1 determinant is expressed only on non-disulfide-linked $\gamma\delta$ TCR.

The function of the $\gamma\delta$ TCR is not known at the present. $\gamma\delta$ TCR using different constant region gene segments may have different functions in T cell differentiation or may be responsive to different antigenic stimuli. Different γ-chain constant region gene segments may be preferentially associated with different variable (Vγ) and joining (Jγ) segments. Identification of such a preferential association will indicate different functions for $\gamma\delta$ TCR using Cγ1, and Cγ2. Additional studies are needed to answer these questions.

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