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Research Article

Early thymus-derived lymphocytes bearing the T gamma gene product in association with the CD3(T3) complex have recently been described. We report a unique case of human acute lymphoblastic leukemia with a CD2+, CD3+, CD4-, CD5+, CD7+, CD8-, WT31- phenotype. These cells were found to have T gamma gene rearrangement and T gamma transcripts in absence of T alpha or T beta rearrangement or transcripts. Immunoprecipitation studies with anti-CD3 antibodies showed a 43-kD protein associated with T3; this 43-kD protein is also precipitated with antiserum raised against synthetic peptides representing the constant region of the putative T gamma protein.

Acute Leukemia Expressing the Gamma Gene Product of the Putative Second T Cell Receptor

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Abstract

Early thymus-derived lymphocytes bearing the Tγ gene product in association with the CD3(T3) complex have recently been described. We report a unique case of human acute lymphoblastic leukemia with a $CD2^+$, $CD3^+$, $CD4^-$, $CD5^+$, $CD7^+$, $CD8^-$, $WT31^-$ phenotype. These cells were found to have Tγ gene rearrangement and Tγ transcripts in absence of Tα or Tβ rearrangement or transcripts. Immunoprecipitation studies with anti-CD3 antibodies showed a 43-kD protein associated with T3; this 43-kD protein is also precipitated with antiserum raised against synthetic peptides representing the constant region of the putative Tγ protein.

Introduction

Antigen recognition is mediated in T lymphocytes by the T cell receptor, identified in some T cells as a heterodimeric glycoprotein comprised of disulfide-linked α- and β- chains that are encoded by nonlinked rearranging genes (1, 2). This receptor is associated on the membrane of human T cells with the T3 molecular complex. The T3 complex consists of two glycosylated proteins, T3γ and T3δ, and a nonglycosylated protein T3ε (3–5). A third gene with considerable homology with Tβ, designated Tγ, has been recently characterized (6). Transcripts encoded by this gene have been detected in cytolytic T lymphocytes and early in T cell ontogeny (7, 8). The characterization of the Tγ product was recently reported in T cells from patients with immunodeficiency syndromes (9) and in a normal thymocyte subpopulation (10). The new peptide was shown to be associated with the normal T3 molecular complex raising the possibility of a putative second T cell receptor (9, 10).

T acute lymphoblastic leukemias (T-ALL)[1] posses cell surface markers that are very similar to those present in subsets of normal thymocytes (11), and this has led to the belief that leukemic cells are clonal expansion of thymocytes arrested at different stages of maturation. Here we describe the characterization of T-ALL that appears to express the putative Tγ protein.

Methods

Patient and cells. Leukemic cells were obtained from the peripheral blood and bone marrow of a 13-yr-old male who presented with leukocytosis, hepatosplenomegaly but no mediastinal mass at diagnosis. Leukemic cells represented 90% of total peripheral blood mononuclear cells and showed a high nuclear/cytoplasmic ratio and evident nucleoli as morphological characteristics. The patient was induced with multiagent chemotherapy and has been in remission for 6 mo.

Viable cells were phenotyped with monoclonal antibodies and analyzed by indirect immunofluorescence using a cytofluorograf Ortho Spectrum III, (Ortho Diagnostic System, Raritan, NJ) or a fluorescence activated cell sorter (FACS IV, Beckton, Dickinson & Co., Mountain View, CA). Antigens, and monoclonal antibodies used in this study included: CD1, CD2, CD4, CD5, CD7, CD8, CD9, CD10, CD19, CD20, CD24, CDw13, CDw14, and HLA-Dr. Specific antigens of the T cell receptor complex were detected with WT31, a monoclonal antibody that detects a framework epitope on the Tα/β heterodimer (12), and OKT3 (13), and Leu 4 (14), antibodies that recognize CD3. A heteroantiserum (anti-Cγ) raised against synthetic peptides representing a sequence of 21 amino acids (residues 136–157) from the constant region of the putative T gamma protein, was used to determine the presence of Tγ peptide on the T-ALL cells (15).

Nonspecific binding via Fc receptors was ruled out by competitive binding studies. Neoplastic cells were incubated with 20% human AB serum and an indirect immunofluorescence assay was then performed to examine the loss of fluorescent staining as compared with a similar assay done in the absence of 20% human AB serum.

Radiolabeling and immunoprecipitation of cell surface antigens. Cell surface iodination was performed with $Na^{125}I$ and catalyzed by lactoperoxidase as described before (16). 2×10^7 cells were disrupted in lysis buffer containing 0.5% Nonidet P-40 (NP-40; Particle Data Laboratories; LTD, Elmhurst, IL), 0.1% sodium dodecyl sulfate (SDS), 2 mM phenylmethylsulfonylfluoride (PMSF), 1% aprotinin (both from Sigma Chemical Co., St. Louis, MO), in 0.9% NaCl and 0.1 M Tris pH 8.1. After lysis and ultracentrifugation the resulting lysate supernatant was preabsorbed with protein A-Sepharose. The lysate was then used directly (for precipitation studies with anti-Leu-4), or denatured (for precipitation studies with anti-Cγ). Denaturation was conducted essentially as described by Brenner et al. (9). Briefly, the lysate was adjusted to 1% SDS, 2 mM dithiothreitol (DTT), and heated at 68°C for 5 min. It was then cooled to room temperature, adjusted to 20 mM iodoacetamide, and diluted with 4 vol of NP-40 lysis buffer containing 1.5% Triton X-100. Native lysate was then immunoprecipitated with anti Leu-4 or control ascitic fluid and denatured lysate was immunoprecipitated with anti-Cγ or normal rabbit serum. Complexes were then isolated using protein A-Se-

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1. *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; PMSF, phenylmethyl sulfonyl fluoride; SSC, standard saline citrate; T-ALL, T cell acute lymphoblastic leukemia; TE, 10 mM Tris, 1 mM EDTA.

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pharose, and resolved by SDS polyacrylamide gel electrophoresis (PAGE). Autoradiography was conducted as previously described (16).

DNA probes. Tβ gene rearrangement and expression was detected with a 770 base pair (bp) complementary (c) DNA constant region probe (17). The Tγ gene was studied with two different probes. The first, Cg, a 600-bp genomic DNA fragment that hybridizes to the constant region of Tγ (18) was used to detect Tγ transcripts. The second, Jg, is a 1-kb genomic DNA fragment that recognizes J sequences in Tγ (19). Tα transcription was detected with Py 1.4, a 1.1 kb cDNA probe that hybridizes to Tα gene (20).

Detection of gene rearrangement. High molecular weight DNA was obtained from 2×10^7 cells using the nuclei extraction technique as described by Bakhshi et al. (21). After phenol/chloroform/isoamylalcohol (25:24:1) and chloroform/isoamylalcohol (24:1) extraction the DNA was ethanol precipitated with 2 M ammonium acetate and resuspended in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.4). DNA concentration was determined by measurement of the OD_{260}/OD_{280} ratios. 10 μg of this DNA was digested to completion using restriction enzymes Eco RI, Bam HI, and Hind III (Bethesda Research Laboratories, Gaithersburg, MD). The resulting fragments were separated by electrophoresis through 0.8% agarose and then transferred from the gel to a nylon membrane (Zetabind, Cuno, Meriden, CT) in 20× standard saline citrate (SSC) using the procedure described by Southern (22). After washing at 65°C in 0.1 × SSC, 0.5% SDS, filters were prehybridized and hybridized as described before (23). The different bands were visualized on autoradiograms after exposure with two intensifier screens for 48 h at −70°C.

RNA extraction and Northern blot analysis. Total RNA was extracted from leukemic cells and HPB-MLT cell line using the proteinase K method as described by Degen et al. (24). 30 μg of total RNA was electrophoresed on 1% agarose-formaldehyde gels and subsequently transferred to nylon membranes in 10× SSC using the same procedure as for DNA transfer. Prehybridization and hybridization conditions were similar as for southern analysis. Transcript sizes were calculated by comparing the RNA migration with ribosomal RNA molecular weight standards.

Results

The neoplastic cells expressed an unusual T cell profile; $CD1^-$, $CD2^+$, $CD3^+$, $CD4^-$, $CD5^+$, $CD7^+$, and $CD8^-$. Markers for B lymphoid, and myeloid lineage were negative; $CD9^-$, $CD10^-$, $CDw13^-$, $CDw14^-$, $CD19^-$, $CD20^-$, $CD24^-$, and HLA-DR^-. Fig. 1 shows the immunofluorescent profile of normal peripheral T cells (*A, C*) and leukemic cells (*B, D*) examined for CD3 (*A, B*) and Tα/β (*C, D*) expression. The leukemic cells were clearly $CD3^+$ (93%, mean channel, 21), although to a lesser degree than normal T cells (81%, mean channel, 68). Leukemic cells did not express the Tα/β heterodimer as detected by WT31 (4%, mean channel, 4. Normal T cells were 71% positive, mean channel, 30). Nonspecific binding of anti-CD3 antibodies via Fc receptors was ruled out with human AB serum as described in Methods. (Data not shown.)

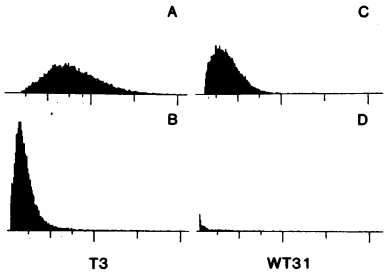

Figure 1. FACS analysis of peripheral blood T cells or patients leukemia cells stained with OKT3 (CD3) or WT31 (Tα/β framework) using indirect immunofluorescence. Background staining has been subtracted from all histograms. Cell number increased from bottom to top on the *y*-axis, fluorescence intensity increased from left to right on the *x*-axis. The *x*-axis is on linear scale.

Southern blot analysis demonstrated a germline Tβ pattern in leukemic cells when compared with placenta DNA. This pattern, shown in Fig. 2 *A*, after Eco RI digestion, was confirmed with Bam Hi and Hind III digestions (data not shown). Analysis of the Tγ gene with Jg, a probe that recognizes two J regions shown two rearranged bands of 4.2 and 2.5 kb (Fig. 2 *B*), corresponding to types IV and V previously described (19, 23).

We next studied the leukemic cells for the presence of RNA transcripts. No Tα/β transcripts were detected in the RNA from the malignant cells, whereas expression of both was clearly detected in RNA from control cells (data not shown). Northern analysis in Fig. 2 *C* shows the distinct presence of a 1.6-kB Tγ transcript in the leukemic cells of the patient.

A radioimmunoprecipitation experiment that was designed to detect the expression of CD3 and associated proteins is shown in Fig. 3. A prominent precipitate between 18 and 30 kD, corresponding to the CD3 complex is clearly visible in normal peripheral T cells (lane *5*). Also seen in lane *5* are bands running at ~ 40 and 49 kD corresponding to the Tα/β heterodimer. Lane *4* shows the results obtained with anti-Leu4 and the T-ALL cells. The CD3 complex was precipitated although, consistent with the FACS histograms in Fig. 1, the amount of CD3 is less than observed with normal T cells. In contrast to normal T cells, the anti-Leu4 precipitate of T-ALL cells also yields a single 43-kD band. To determine whether the 43-kD protein shown in lane *4* might be similar to the putative Tγ protein, immunoprecipitation with anti-Cγ antiserum was performed. An intense precipitate is seen in lane 1 running at 43 kD, with a mobility identical to the protein co-precipitated with anti-Leu4 in lane *4*.

Discussion

The Tγ gene was initially described in murine T cells (6) and subsequently in human T cells (18). The presence of Tγ tran-

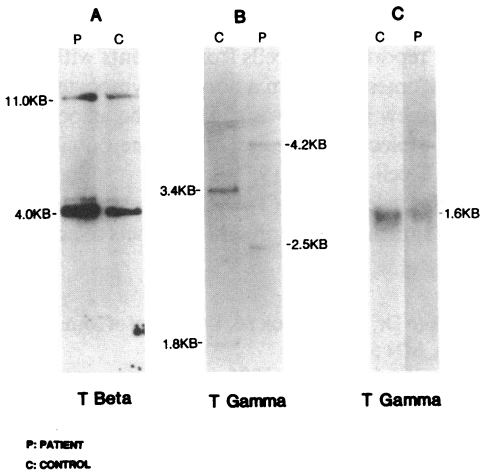

Figure 2. DNA and RNA analysis of leukemic cells. (*A*) EcoRI digest probed with Tβ. (*B*) EcoRI digest probed with T gamma Jg. (*C*) Total RNA probed with T gamma C. (*P*, patient; *C*, control.) Control in lanes A and B, peripheral T lymphocytes. Control in lane C, HPB-MLT.

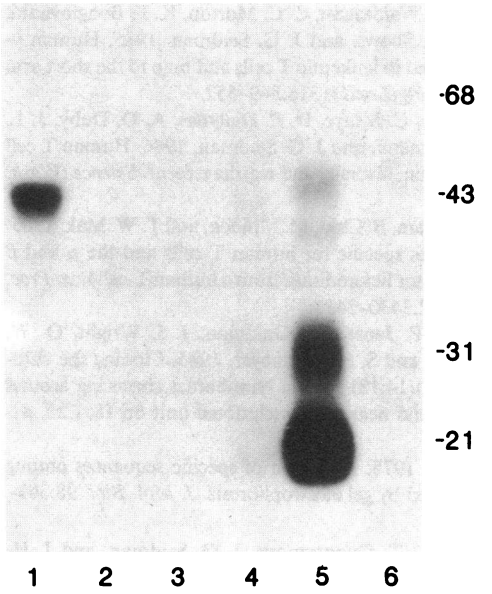

Figure 3. Immunoprecipitation of iodinated surface molecules from leukemic and normal T cells using antibodies to the T cell receptor complex. Lanes *1–4*, leukemic cells. Lanes *5, 6*, peripheral blood T cells. Lane *1*, anti-Cγ. Lane *2*, normal rabbit serum. Lanes *3* and *6*, control ascitic fluid. Lanes *4, 5*, anti-CD3 (Leu4). Molecular mass markers, in kilodaltons, are shown on the *y*-axis. The gel was electrophoresed under reducing conditions. Immunoprecipitation in lane *4* is weaker than that in lane *5* reflecting differences in CD3 expression between normal peripheral T cells and leukemic cells and the presence of more free iodine in the immunoprecipitate.

scripts in thymic precursors (but less frequently in mature cells) suggested that the gene product could be functional early in thymic development (8). The recent demonstration of the Tγ gene product on the surface of cells from patients with immunodeficiency syndromes (9) and in developing normal thymocytes (10) is consistent with this hypothesis.

Here we describe the first example of fresh T-ALL cells with the $CD3^+$, $CD4^-$, $CD8^-$, $WT31^-$ phenotype that appear to express the Tγ gene product on their cell surface. These cells expressed the CD3 protein complex as demonstrated by immunofluorescence and immunoprecipitation studies. However, when studied for expression of the Tα/β heterodimer,the cells showed no reactivity with the antibody WT31, which recognizes a framework epitope onthe Tα/β heterodimer (12). Immunoprecipitation studies under reducing conditions with an anti-CD3 antibody showed the normal CD3 complex and a unique 43-kD protein in these cells. When immunoprecipitated with anti-Cγ antiserum, these cells also demonstrated the 43-kd protein. This finding, along with the existence of Tγ transcripts, suggests that the new CD3 associated protein present in these neoplastic cells correspond to the Tγ gene product.

Biochemical analysis of the putative Tγ receptor reported by various groups have shown differences in the protein size. Brenner et al. (9) studied cells from patients with immunodeficiency and described two different CD3 associated proteins: one, of 55 kD, was immunoprecipitated with anti-Cγ antiserum, the second a 40-kD protein was not precipitated with anti-Cγ. Bank et al. (10) examined thymocytes and found a heterodimer consisting of a 60-kD protein associated with a 44-kD chain recognized by a Cγ antiserum. Recently, Weiss et al. (25) and Moingeon et al. (26) described two additional 55–60 kD and 44 kD protein associated with CD3. Both these studies showed the presence of Tβ transcripts, and the relationship of these to Tγ is not clear since a Tγ antiserum were not used in these studies.

The leukemic cells described herein express a unique 43-kD protein associated with CD3 that is precipitated with a Tγ antiserum. However, no Tβ or Tα transcripts were detected in these cells. Moreover, our study indicates that this T-ALL has Tγ rearrangements similar to that described previously in leukemic and normal cells (19, 23).

Leukemic cells are thought to represent clonal populations arrested at different stages of hemopoiesis. These cells generally retain fidelity to a differentiation-linked phenotype although exceptions have been described (27, 28). To our knowledge, this is the first report of a leukemic cell presenting the unusual $CD3^+$, $CD4^-$, $CD8^-$, $WT31^-$ phenotype that expressed a protein other than the Tα/β heterodimer associated with CD3. The precipitation of this protein by an anti-Cγ antiserum indicates that this protein is the product of the Tγ gene. Further functional, biochemical, and cell culture studies will be done to determine if these cells correspond to any functional subset of immunocompetent T cells and to further define the function of this subset.

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