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Research Article

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Phenotypic Conversion of T Lymphoblastic Lymphoma to Acute Biphenotypic Leukemia Composed of Lymphoblasts and Myeloblasts

Molecular Genetic Evidence of the Same Clonal Origin

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Abstract

Acute biphenotypic leukemia composed of lymphoblasts and myeloblasts developed in a patient with T lymphoblastic lymphoma (T-LBL) who had an anterior mediastinal mass. A novel myeloid cell line, termed TK-1, has been established from his peripheral blood after the leukemic conversion. The identical rearranged pattern of T cell receptor γ -chain gene was observed among the DNAs derived from lymph node cells in the lymphoma phase, the myeloid cell line TK-1, and the subclones with different karyotypes (TK-1B and TK-1D), which showed that myeloid cells had been derived from the T-LBL of the same patient. This finding demonstrates that phenotypic conversion occurs in the clonally propagating tumor cells and suggests that some hematopoietic cells retain the capacity to adopt either lineage.

Introduction

Development of acute myeloblastic leukemia (AML)¹ in patients with T-lymphoblastic lymphoma (T-LBL) (1) has been reported only rarely (2–4), and the clonality of T-LBL and AML has been the unsettled, central problem. We established the cell line TK-1 (5) from a patient with biphenotypic leukemia whose initial clinical feature was T-LBL with anterior mediastinal mass. Although it was suggested that a switch from lymphoid to myeloid lineage may occur in the leukemia (6–11), the same clonality in the molecular level has not been proved. In our case, both T-LBL and myeloid cells had the germ line configuration of immunoglobulin heavy chain (IgH) gene and T cell receptor (TcR) β -chain gene. From the analysis of rearrangement of the TcR γ -chain gene, we are now able to demonstrate the molecular genetic evidence that shows the phenotypic conversion from the original T-LBL cells to myeloid cells did actually occur in the same clonal hematopoietic cells.

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1. Abbreviations used in this paper: AML, acute myeloblastic leukemia; IgH, Ig heavy chain; TcR, T cell receptor; T-LBL, T cell lymphoblastic lymphoma.

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Methods

Patient. An abrupt leukemic conversion occurred (first leukemic phase) in a 22-yr-old male with T-LBL (CD7, 93%; CD13, 0%; details are shown in Table I) and an anterior mediastinal mass. The leukemic cells of peripheral blood were composed of lymphoblasts (58.5%, FAB-L2 [12]) and myeloblasts (27.5%, FAB-M4). The latter had prominent azurophilic granules and were positive for peroxidase, nonspecific esterase, and chloroacetate esterase stainings, simultaneously. Both blasts disappeared transiently after treatment with a combination of vincristine, a large dose of methotrexate, and L-asparaginase. Details were described elsewhere (5). 3 mo later, a second leukemic phase developed with lymphoblasts only, and the patient died of respiratory failure. 2'-Deoxycoformycin was not used during the clinical course.

Cells. Mononuclear cell fractions were obtained from a lymph node by mincing and elimination of aggregates after sedimentation, or they were separated from the peripheral blood by Ficoll-Hypaque density gradient centrifugation.

Cell cultures. TK-1 was established from his peripheral blood in the first leukemic phase. Then the cells were subcloned by a limiting dilution method, and TK-1B and TK-1D were obtained. The karyotype of TK-1B was pseudodiploid, having a complex translocation involving 14, 17, and unknown chromosomes, while TK-1D retained the normal male karyotype. The parental TK-1 cell line appears to be a mixture of both subclones by karyotype analysis.

Immunological characterization. Surface marker analysis was performed by using immunofluorescence as described previously (13) (Table I).

Analysis of DNA. High molecular weight DNA was extracted from frozen cell suspensions, as described previously (14). 3 μ g of DNA was digested with restriction enzyme Eco RI, Hind III, Bam HI, or Xba I; then separated by electrophoresis on 0.6% agarose gel; transferred (15) to nitrocellulose by 10 \times standard saline citrate; hybridized at 65°C to the ³²P-labeled human DNA probe; washed at the appropriate stringency, and autoradiographed.

Probes. J γ 1, a Hind III-Eco RI fragment that recognizes both joining regions of TcR γ -chain gene (16) (J γ 1 and 2); C β 1, a Hind III-Eco RI fragment that recognizes both constant regions of TcR β -chain gene (17) (C β 1 and 2); JH, an Eco RI-Hind III fragment that recognizes a joining region of the IgH gene (18).

Results

As shown in Fig. 1, cells from a lymph node in the LBL stage, TK-1, TK-1B, TK-1D, and cells from peripheral blood in the second leukemic phase, showed the same rearranged pattern of the TcR γ -chain gene when digested by Eco RI or Hind III. In Eco RI digestion, one allele had the rearranged J γ 1 gene which produced a new band at the 2.3-kb position while the other allele remained in the germ line. The Eco RI digest of the EBV-transformed cell line, which was derived from the same patient, represents the germ line configuration of TcR γ -chain

Table I. Characteristics of the Original Lymph Node Cells and Established Cell Lines

	Lymph node	TK-1	TK-1B	TK-1D
Peroxidase	-	++	++	++
CD2	-	-	-	-
CD7	++	-	-	-
CD3,4,6,8	-	-	NT	NT
CD11	-	-	-	-
CD13	-	-	-	+
CD14	NT	-	-	-
CD15	NT	+	-	-
Ia	+	++	++	++
CD10	-	-	-	-
CD20	-	-	-	-
TdT	++	-	-	-
Karyotype	No adequate metaphase	46,XY, (12 cells) 46,XY,14q-,17q+ (14 cells)	46,XY,14q-,17q+	46,XY

-, <15% positive cells; +, >15% positive cells; ++, >40% positive cells; NT, not tested. * LeuM1.

gene with two bands of 3.3 kb ($J\gamma_2$) and 1.8 kb ($J\gamma_1$), thus excluding the possibility of the polymorphism of the TcR γ -chain gene in this patient. Hind III digests were also identical to the rearranged pattern of the TcR γ -chain gene in these five samples; i.e., the rearranged $J\gamma_1$ of one allele was detected at the 3.7-kb position with the other, nonrearranged $J\gamma$ segments (with $J\gamma_1$ of another allele, 2.1-kb fragment; and $J\gamma_2$ of each allele, 2.1-kb and polymorphic [16] 4.5-kb fragments, respectively). Further, evidence that the same rearranged pattern occurs in Bam HI digestion (data not shown) supports the

proposition that these cells have the same clonal origin. These five samples retained the germ line configuration of the IgH gene and the TcR β -chain gene when digested by two different restriction enzymes (Figs. 2 and 3).

Discussion

Novel human myeloid cell line TK-1, and the subclones TK-1B and TK-1D, were established from the biphenotypic leukemic cells in the patient with T-LBL. The existence of

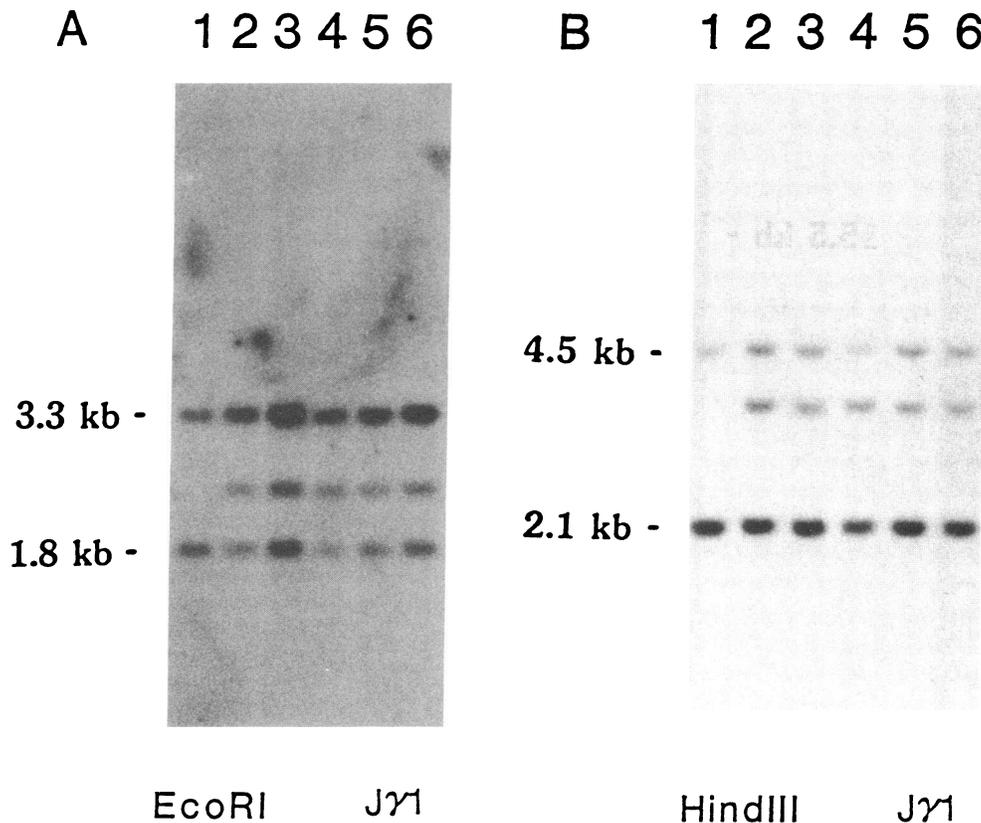


Figure 1. Southern blotting hybridization of the TcR γ -chain gene ($J\gamma_1$) probe to Eco RI (A) or Hind III (B) digested DNA from the same patient. Cells from a lymph node in lymphoma phase (lane 2), novel myeloid cell line TK-1 derived from peripheral blood after the leukemic conversion (lane 3), the subclones TK-1B (lane 4) and TK-1D (lane 5) with different karyotypes, and cells from peripheral blood in the second leukemic phase (lane 6), all showed a pattern identical to the rearranged pattern of TcR γ -chain gene, while EBV-transformed cells derived from the same patient (lane 1) showed the germ line configuration of TcR γ -chain gene, excluding the polymorphism of this gene in this patient.

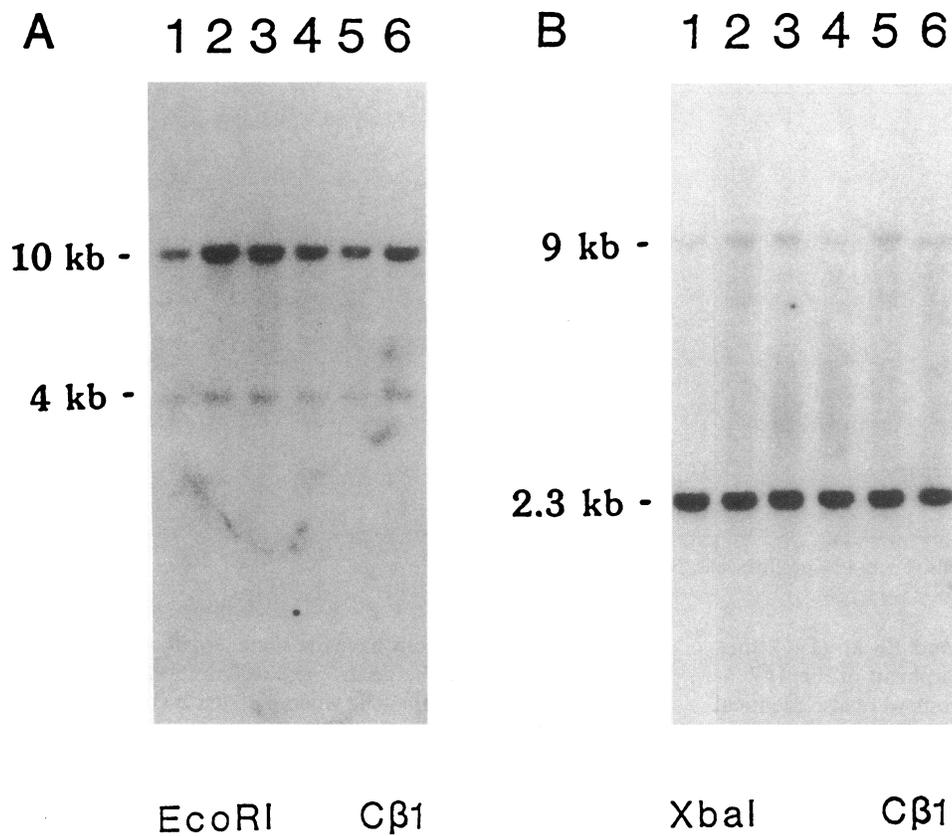


Figure 2. Southern blotting hybridization of the TcR β -chain gene (C β 1) probe to Eco RI (A) or Xba I (B) digested DNA. Identification numbers over lanes correspond to those in the legend of Fig. 1. All samples had the germ line configuration of TcR β -chain gene.

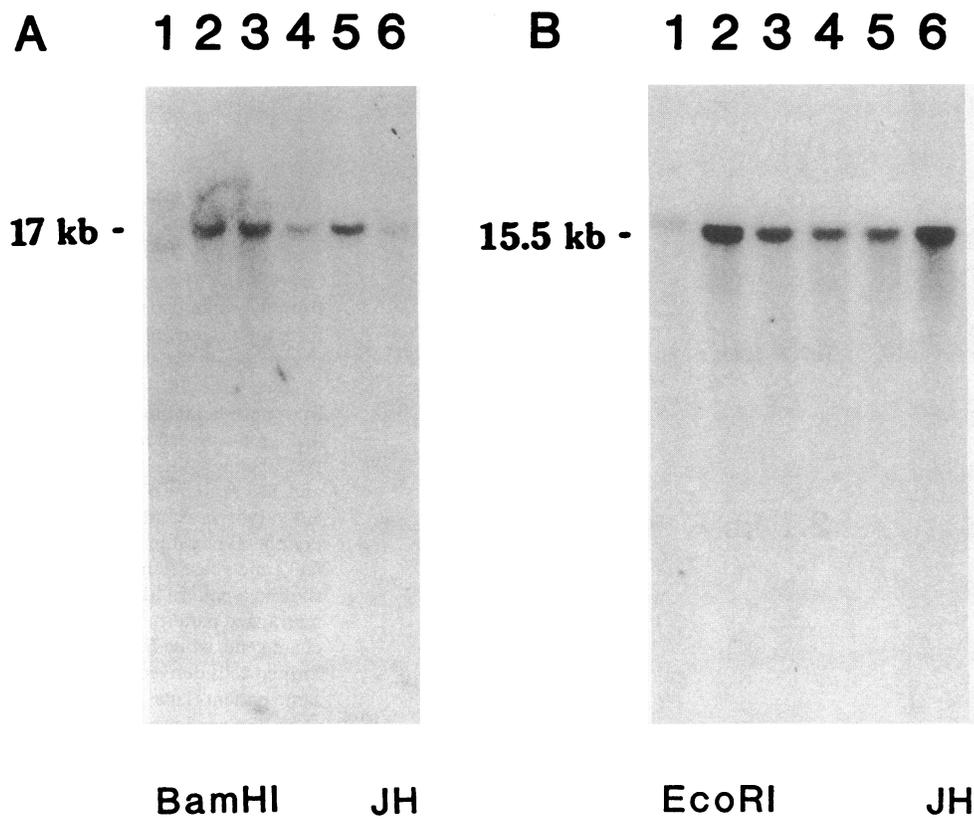


Figure 3. Southern blotting hybridization of the IgH gene (JH) probe to Bam HI (A) or Eco RI (B) digested DNA. Identification numbers over lanes correspond to the same samples as in Fig. 1. DNA derived from the same patient (except lane 1) showed the same nonrearranged configuration of JH gene (their sizes are given in this figure), while EBV-transformed cells derived from the same patient showed at least one detectable rearranged band.

both peroxidase positive cells and lymphoid ones in the same patient raises an important issue: a lineage switch may occur in clonally proliferating, hematopoietic cells from the same origin. It was not possible to solve this problem until we analyzed the TcR γ -chain gene rearrangement, because TK-1B and TK-1D had neither common karyotypic abnormality nor distinguishable molecular genetic markers, which included rearrangements of TcR β -chain gene and IgH gene (19, 20). Only the analysis of the TcR γ -chain gene made it possible to prove the same clonality in those tumor cells and cell lines that were derived from the same patient. The original T-LBL cells were phenotypically classified as the earliest stage in T cell ontogeny (21-23), and the peroxidase positive cells appeared in the patient's blood with the frozen state of rearrangement of the TcR γ -chain gene. This indicates the lineage switch among the population of tumor cells during the process of leukemic conversion. In certain hematopoietic microenvironments, genes may be abnormally activated to induce a myeloid appearance, or the differentiating potential of T cell lineage may be decreased. After the leukemic conversion, the patient was treated with chemotherapy protocol for acute lymphoblastic leukemia because the population of lymphoblasts exceeded that of myeloblasts, and a transient remission was achieved. In the second leukemic phase, however, only lymphoblasts arose without myeloid markers. This may be explained in one of the following ways: either the original lymphoblasts reappeared after the acute lymphoblastic leukemia chemotherapy to which only myeloid leukemic cells responded, or, alternatively, myeloid leukemic cells were converted again to lymphoblasts. If the progenitor leukemic cells preserve a bidirectional differentiation potential (myeloid and lymphoid lineages) (13), then the hematopoietic environment created by chemotherapy may have caused the cells to be unidirectional at the second leukemic stage. Recent advances (24, 25) of the stem cell biology strongly suggest that heterogeneous stem cells have a potential to differentiate stochastically into any lineage at the primitive stage of normal hematopoiesis. Phenotypic interconversion between myeloid and lymphoid lineages, observed in our report, may reflect this stochastic model in part. Although it is difficult at present to prove the stochastic model by normal pluripotent stem cells in vitro, the cell lines reported on here and those found in other studies (26) may serve as tools to study the mechanism of phenotypic alteration, lineage conversion, and promiscuity (27) in the hematopoietic system.

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