

Detection of clonotypic IGH and TCR rearrangements in the neonatal blood spots of infants and children with B-cell precursor acute lymphoblastic leukemia

Tomohito Yagi, Shigeyoshi Hibi, Yasuhiro Tabata, Kikuko Kuriyama, Tomoko Teramura, Tetsuo Hashida, Yoshitaka Shimizu, Tetsuya Takimoto, Shinjiro Todo, Tadashi Sawada, and Shinsaku Imashuku

An attractive hypothesis is that in utero exposure of hematopoietic cells to oncogenic agents can induce molecular changes leading to overt acute lymphoblastic leukemia (ALL) in infants and perhaps older children as well. Although supported by studies of identical infant twins with concordant leukemia, and of nontwined patients with *MLL* gene rearrangements, this concept has not been extended to the larger population of B-lineage ALL patients who lack unique non-

constitutive mutations or abnormally rearranged genes. We therefore sought to demonstrate a prenatal origin for 7 cases of B-cell precursor ALL (either CD10⁺ or CD10⁻) that had been diagnosed in infants and children 14 days to 9 years of age. Using a polymerase chain reaction-based assay, we identified the same clonotypic immunoglobulin heavy-chain complementarity determining region or T-cell receptor V_D2-D_D3 sequences in the neonatal blood spots (Guthrie card) and leukemic cell

DNAs of 2 infants with CD10⁻ ALL and 2 of the 5 older patients with CD10⁺ ALL. Nucleotide sequencing showed a paucity of N or P regions and shortened D germ line and conserved J sequences, indicative of cells arising from fetal hematopoiesis. Our findings strongly suggest a prenatal origin for some cases of B-cell precursor ALL lacking specific clonotypic abnormalities. (Blood. 2000;96:264-268)

© 2000 by The American Society of Hematology

Introduction

B-cell precursor acute lymphoblastic leukemia (ALL) is by far the most common form of leukemia in children. Its incidence is highest between 1 and 5 years of age, with a peak between 2 and 4 years.^{1,2} Recent studies of identical twins with concordant ALL established that leukemic transformation in these cases was initiated during the fetal period,³⁻⁶ which would help to explain the common phenotypes, karyotypes, and clonal gene rearrangements that typify the leukemic cells of such patients. This finding was interpreted as evidence for in utero leukemic conversion in one twin, with the transformed cells migrating to the other twin through the placental circulation.⁵ In 1997, Gale et al⁷ demonstrated *MLL-AF4* gene fusion in neonatal blood spots (Guthrie card) of nontwined patients who developed acute leukemia at the ages of 5 months to 2 years. Taken together, these observations provide compelling support for the in utero origin of some cases of childhood ALL. However, approximately 50% of childhood ALL patients, most with leukemic B-cell precursors, lack abnormal gene rearrangements that could be used to backtrack their leukemias to the fetal stage of development.

We therefore analyzed the Guthrie card blood spots of infants and children who were diagnosed with either CD10⁺ or CD10⁻ ALL at 14 days to 9 years of age (median, 2 years), using a combination of nonpathologic immunoglobulin heavy chain (IGH) and T-cell receptor delta (TCRD) gene rearrangements to identify individual leukemic clones. This polymerase chain reaction (PCR) strategy was based on the high frequency of such changes in B-lineage ALL (95% IGH, 60% to 70% TCRD)⁸⁻¹⁴ and the uniqueness of somatic mutations within complementarity determin-

ing region 3 (CDR3) of the IGH gene.^{15,16} CDR3 comprises the 3' end of V_H, all of D, and the 5' end of J_H; it also contains randomly inserted nucleotides. The TCRD gene contains a limited number of functional V and J segments, but rearrangements affecting V_D2 and D_D3 are relatively common in B-lineage ALL, prompting us to include primers for these 2 regions in our PCR analysis. Here we report clonotypic IGH and TCRD gene rearrangements in the Guthrie card blood spots of 4 patients with either CD10⁺ or CD10⁻ B-lineage ALL.

Patients, materials, and methods

Patients and controls

We studied the leukemic bone marrow cells and Guthrie cards of 7 pediatric patients with B-cell precursor ALL after obtaining informed consent from the patients' parents or guardians. The clinical and laboratory features of these patients are summarized in Table 1. Two of the cases developed in infants (CD10⁻, CD19⁺, and HLA-DR⁺) and 5 in children (CD10⁺, CD19⁺, and HLA-DR⁺). The median age at diagnosis in the latter group was 2 years (range, 1.5 to 9 years). The leukocyte counts on admission ranged from 2300 to 388 000/μL (median, 121 600/μL). Both of the infants had *MLL* gene rearrangement by Southern blot analysis (data not shown). It was confirmed that the 5 CD10⁺ patients had no infectious symptoms at the time of neonatal blood spot collection. Bone marrow (n = 2) and

From the Department of Pediatrics, Kyoto Prefectural University of Medicine, Kyoto, Japan; the Department of Pediatrics, Otsu Red Cross Hospital, Otsu, Japan; and Kyoto City Institute of Health and Environmental Sciences, Kyoto, Japan.

Submitted April 21, 1999; accepted February 20, 2000.

Reprints: Tomohito Yagi, Department of Pediatrics, Kyoto Prefectural Univer-

sity of Medicine, 602-8566, Kajicho 465 Hirokoji Kamigyoku, Kyoto, Japan; e-mail: yagi@ped.kpu-m.ac.jp.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

© 2000 by The American Society of Hematology

Table 1. Clinical data on patients with CD10⁺ or CD10⁻ B-precursor ALL

Case no.	Sex	Age at onset*	Leukocyte count (per μ L)	Percentage of blasts in peripheral blood†	Karyotype	Positive immunological markers‡
1	F	14 d	121 600	68	46XX,t(4;11;15)	19, 34, 45, 79a, HLA-DR
2	M	2 mo	257 000	93	46XY,t(4;11)(q21;q23)	15, 19, 34, 45, 79a, HLA-DR
3	M	2 y	67 900	87	46XY	10, 19, 33, 34, HLA-DR
4	M	2.2 y	159 000	90	63XXY	10, 19, 20, 34, 45, HLA-DR
5	M	1.5 y	388 000	88	46XY,t(11;12)(q13;q24)	10, 19, 34, 45, HLA-DR
6	M	9 y	6 500	30	46XY	10, 19, 20, 34, HLA-DR
7	M	2 y	2 300	0	46XY	10, 19, 20, 22, 34, HLA-DR

*d indicates days; mo, months; y, years.

†Case 7 had 98% lymphoblasts in 2 successive bone marrow aspirates.

‡An *MLL* fusion gene was demonstrated in cases 1 and 2 by Southern blot analysis (not shown).

peripheral blood (n = 15) samples from healthy infants, children, and adults and Guthrie cards of the same-aged patients (n = 8) who did not develop leukemia were obtained with informed consent and served as normal controls.

Specimens

DNA was extracted from the leukemic bone marrow cells that had been stored in liquid nitrogen since diagnosis and from control peripheral blood and bone marrow cells (DnaQuick DNA extraction kit; Dainippon Pharmaceutical Co, Osaka, Japan). The Guthrie cards had been stored either at room temperature or at -80°C at several different laboratories. Samples cut from each Guthrie card (3 mm \times 3-mm squares containing the equivalent of about 1×10^4 mononuclear cells) were extracted in 30 μ L of double-distilled water for 2 hours at 55°C . The resultant eluates were directly amplified with the use of the Ampdirect PCR kit (Shimadzu Co, Tokyo, Japan). Successful DNA extraction was confirmed by demonstration of the amplified beta-globin gene in all experiments.

In preliminary sensitivity experiments, the starting aliquot containing 1×10^5 leukemic cells added to 1 mL blood (cell count, 1×10^7) obtained from a healthy adult was sequentially diluted at concentrations ranging from 10^{-1} to 10^{-6} /mL. These samples were then used to determine the limit of detection of the PCR products amplified from extracted leukemic cell DNA as well as material eluted from the Guthrie cards. In the latter situation, the analyses were performed with cards on which a 50- μ L droplet of blood containing the diluted leukemic cells had been stored for at least 2 months prior to extraction.

PCR analysis using consensus primers

Samples of DNA (100 ng) from leukemic or nonleukemic peripheral blood and bone marrow cells were subjected to PCR with the use of consensus primers for rearranged IGH and TCRD regions.^{11,17,18} The primers for the IGH CDR3 region were FR3A (5'-ACACGGC(C/T)(G/C)TGTATTACTGT-3'), LJH (5'-TGAGGAGACGGTG-ACC-3'), and VLJH (5'-GTGAC-CAGGGTNCCTTGGCCCCAG-3'). The primers for the TCRD region were V_D2E (5'-GAGTCATGTCAGCC-ATTGAG-3') and D_D3E (5'-AGGAAATGGCACTTTTGGC-3') for the first PCR reaction, and V_D2I (5'-GCACCATCAGAGAGATGA-3') and D_D3I (5'-TTGTAGCACTGT-GCGTATCC-3') for nested PCR. The amplification conditions for each targeted segment were as follows: 1 \times buffer (25 mmol/L Tris buffer, pH 9.3 containing 50 mmol/L KCl, 2 mmol/L MgCl₂, 1 mmol/L 2-mercaptoethanol), 200 μ mol/L dNTP, 0.5 μ mol/L primers, and 2.5 units of ExTaq (Takara Shuzo Co, Shiga, Japan) in a final reaction volume of 50 μ L. The cycling conditions were 1 minute at 92°C , 45 seconds at 62°C (annealing temperature), and 1 minute at 72°C for a total of 35 cycles followed by a final incubation period at 72°C for 5 minutes. (Perkin-Elmer Gene Amp 2400; Perkin-Elmer Cetus, Norwalk, CT). The annealing temperature was reduced by 0.2°C per cycle from 62°C to 55°C . These amplifications were performed on either a Perkin-Elmer Gene Amp 2400 or a Perkin-Elmer 9700 instrument.

PCR analysis using allele-specific primers

For cases 5, 6, and 7, in which leukemic clone with same sequence was not amplified in Guthrie cards, we set up the allele-specific primers for the

rearranged IGH CDR3 regions of leukemic cells for each case. The primer sequences used were 5'-CCT TATTACTATGGTTCGGGGAGTTATA-3' for case 5, 5'-CCGGATTACAGCAGCTCGTCC-3' for case 6, and 5'-GAGATCTGGTAGTACTACTTTGTTGA-3' for case 7, respectively, and nested PCR was performed by using FR3A, LJH, and each allele-specific primer. The cycle conditions were the same as previously described, except for case 7, in which the annealing temperature was reduced from 60°C to 52°C . Similarly, we attempted to set up the allele-specific primers in the rearranged TCRD regions for these cases, but this was not possible because of a paucity of N region.

Cloning and sequencing

Amplified PCR products of the rearranged IGH CDR3 or TCRD region were cloned by ligation with the use of a pCR2.1 TA cloning kit (Invitrogen, Carlsbad, CA). At least 15 independent colonies were picked and expanded (Table 2). The plasmids were purified with a DNA purification kit (Promega, Madison, WI), and sequencing was performed on a mixture containing 8 μ g of DNA, 2 μ L each of dNTP reagents from the Thermo Sequenase fluorescent labeled primer cycle sequencing kit containing 7-deazo-deoxyguanosine triphosphate (dGTP) (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and 2 μ L of 1.5 pmol of upstream or understream primers. Sequencing was performed under the following conditions: 92°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds for 30 cycles in an ALF DNA sequencer (Pharmacia, LKB Biotech, Piscataway, NJ). The sequence data were assigned on the basis of their similarity to BLAST sequences and confirmed by homology to each germ-line sequence.¹⁹⁻²²

Results

Limiting dilution experiments

The sensitivity of our PCR analysis was studied both with DNA extracted from serially diluted leukemic bone marrow cells and

Table 2. Ratio of colony numbers showing identical nucleotide sequences per picked-up colonies

Case	IGH		TCRD	
	Guthrie card	Leukemic cell	Guthrie card	Leukemic cell
1	14/15 (93.3)	15/16 (93.8)	13/15 (86.7)	12/15 (80.0)
2	11/17 (64.7)	13/15 (86.7)	NA	NA
3	13/18 (72.2)	15/21 (71.4)	8/19 (42.1)	11/16 (68.8)
4a	14/21 (66.7)	13/16 (81.3)	6/15 (40.0)	10/18 (55.6)
4b	9/16 (56.3)	NA	NA	NA
5	NA	12/16 (75.0)	NA	NA
6	NA	8/15 (53.3)	NA	10/17 (58.8)
7	NA	13/16 (81.3)	NA	12/18 (66.7)

NA indicates not amplified; parentheses contain percentages; IGH indicates immunoglobulin heavy chain; TCRD, T-cell receptor delta. The rearranged IGH and TCRD regions were amplified with the use of consensus primer pairs (see text). The nucleotide sequences determined are shown in Table 3 and 4.

Table 3. Comparison of nucleotide sequences in rearranged IGH CDR3 leukemic cell DNA (L) and corresponding Guthrie card DNA (G)*

Case	V	N	D	N	J	D _H	J _H
1(G)	TGTGCAAGAGATCCC	c	TAGTAGTACCAGCT	cgtcc	TTACTACTACTACATGGAC	Dir-4b	J6
1(L)	TGTGCAAGAGATCCC	c	TAGTAGTACCAGCT	cgtcc	TTACTACTACTACATGGAC		
2(G)	TGTGCAAGAGATCC		GTATAGCAGCTCGTCC	ccaa	TACTACTACATGGAC	DN4	J6
2(L)	TGTGCAAGAGATCC		GTATAGCAGCTCGTCC	ccaa	TACTACTACATGGAC		
3(G)	CAGGGGTCCGTATTTT		TATGGTTCG	cceg	CTGGTTCGACCCCTGG	Dxp'-1	J5
3(L)	CAGGGGTCCGTATTTT		TATGGTTCG	cceg	CTGGTTCGACCCCTGG		
4a(G)†	TGTGCAAGAGATCCC	aaggat	TAGCAGCTCG	cccct	TTACTACTACATGGAC	DN4	J6
4a(L)	TGTGCAAGAGATCCC	aaggat	TAGCAGCTCG	cccct	TTACTACTACATGGAC		
4b(G)	TGTGCGAGAGATC		ATTACGATATTTTGGACTGGTTATTA		ACTGGTTCGACCCCTG	Dxp-1	J5
4b(L)			not amplified				
5(G)			not amplified				
5(L)	TGTGCAAGAGA TCCC	cct	TATTACTATGGTTCGGGGAGTTATTATTAC	gttatta	CTACTTTGACTACTGG	Dxp'-1	J4
6(G)			not amplified				
6(L)	TGTGCAAGAGATCCC	ccggattc	AGCAGCTCGTCC	cc	TTACTACTACTATGGAC	DN4	J6
7(G)			not amplified				
7(L)	TGTGCGAGATC	tgg	TAGTACTACTTT	g	TTGACTACTGGGCCAA	Dir-5	J6

*The findings are categorized by IGH region (V, N, D, and J). N-region sequences are given in lowercase letters. Germ-line sequences are identical to those reported in the literature.¹⁹⁻²²

†Biallelic amplification (4a, 4b) for Guthrie card DNA, but not for leukemic cell DNA (4a only).

with material directly eluted from experimentally prepared blood spots on Guthrie cards, following previously described methods.⁷ Analysis of leukemic cell DNA for rearrangements in the IGH CDR3 region indicated a detection limit of 1 leukemic cell per 1000 normal cells (10^{-3}) (Figure 1A). A similar limit was established for DNA eluted from Guthrie cards (Figure 1B). In the PCR assay for rearrangements in the TCRD region, using serial dilutions of leukemic bone marrow cells, we found a detection limit of 1 leukemic cell per 10 000 normal cells, which was 1 log more sensitive than the result for Guthrie card blood spots (10^{-3}) (Figure 1C,D).

PCR analysis of bone marrow, peripheral blood cell, and Guthrie card DNA from normal controls

In the normal controls, DNA obtained from bone marrow, peripheral blood cells, and Guthrie cards did not produce the clonally amplified products in a diffuse smear of IGH and TCRD amplification (data not shown).

PCR analysis of leukemic cell and Guthrie card DNA. In the 2 infants and in all 5 of the remaining patients, the amplified bands of rearranged IGH CDR3 DNA from leukemic cells varied in size from 90 to 120 base pairs (bp), while those of the TCRD region ranged from 70 to 100 bp by electrophoresis. All amplified segments showed a single rearranged allele, which was subcloned

and sequenced. In cases 2 and 5, it was not possible to amplify the targeted TCRD region (Tables 3 and 4).

Using consensus primers for the IGH CDR3 region, we succeeded in amplifying Guthrie card DNA both from infant cases and from 2 of the 5 remaining cases. A single band of rearranged TCRD DNA was visible by agarose gel electrophoresis in cases 1, 3, and 4 (Figure 2). Case 4 also had biallelic bands of amplified IGH CDR3 DNA (Figure 2). As with the DNA from leukemic bone marrow cells in cases 2 and 5, we were unable to amplify the targeted TCRD segments in Guthrie card eluates representing cases 2, 5, 6, and 7. In cases 5, 6, and 7, even with the allele-specific primers in nested PCR analysis, none of the cases demonstrated the clonal amplification.

Summary of cloning sequencing results. As summarized in Table 2, the ratio of colony numbers showing identical nucleotide sequences per picked-up colonies in leukemic cells was in a range of IGH (53.3% to 93.8%) and TCRD (55.6% to 80.0%). In Guthrie cards, we identified identical nucleotide sequences as that of leukemic cells in frequencies of IGH (56.3% to 93.3%) and TCRD (40.0% to 86.7%). Tables 3 and 4 also summarize the sequencing data for the 7 leukemia patients. In 4 cases, the IGH CDR3 findings in Guthrie card and leukemic bone marrow cell DNAs were identical, as were the TCRD results in 3 cases. In case 4, biallelic

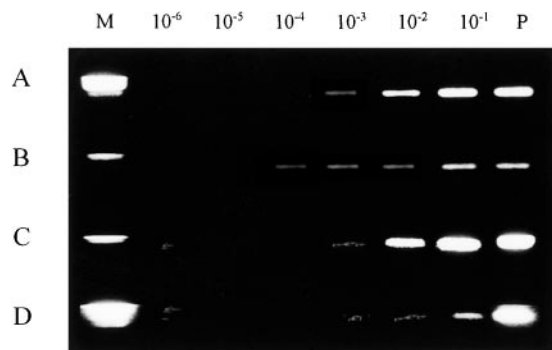


Figure 1. Limiting dilution experiments for rearranged IGH and TCRD. Rearranged IGH and TCRD amplified segments are shown by ethidium bromide gel staining. (A) IGH, leukemic cell DNA. (B) TCRD, leukemic cell DNA. (C) IGH, Guthrie card DNA. (D) TCRD, Guthrie card DNA. Standard limiting dilution (10^{-1} through 10^{-6}) was prepared with positive control cells. The limit for detecting the IGH rearrangement was approximately 10^{-3} compared with 10^{-4} for TCRD in leukemic cells DNA. M indicates DNA size marker; P, amplified product from 100 ng positive control DNA; and N, amplified product from negative control DNA.

Table 4. Comparison of nucleotide sequences of the V_D2-D_D3 rearrangements in leukemic cell DNA (L) and corresponding Guthrie card DNA (G)*

Case	V _D 2	P	N	P	D _D 3
GL	GGT CTT ACT GTG CCT GTG ACA CC				ACT GGG GGA TAC G
1(G)	GGT CTT ACT GTG CCT GTG ACA CC	gt			ACT GGG GGA TAC G
1(L)	GGT CTT ACT GTG CCT GTG ACA CC	gt			ACT GGG GGA TAC G
2(G)				not amplified	
2(L)				not amplified	
3(G)	GGT CTT ACT GTG CCT GTG ACA CC	ta			ACT GGG GGA TAC G
3(L)	GGT CTT ACT GTG CCT GTG ACA CC	ta			ACT GGG GGA TAC G
4(G)	GGT CTT ACT GTG CCT GTG ACA	a			GGA TAC G
4(L)	GGT CTT ACT GTG CCT GTG ACA	a			GGA TAC G
5(G)				not amplified	
5(L)				not amplified	
6(G)				not amplified	
6(L)	GGT CTT ACT GTG CCT GTG ACA CC	g			T GGG GGA TAC G
7(G)				not amplified	
7(L)	GGT CTT ACT GTG CCT GTG A	gt			ACT GGG GGA TAC G

*See footnotes to Table 3 for additional information. P indicates P region; N, N region.

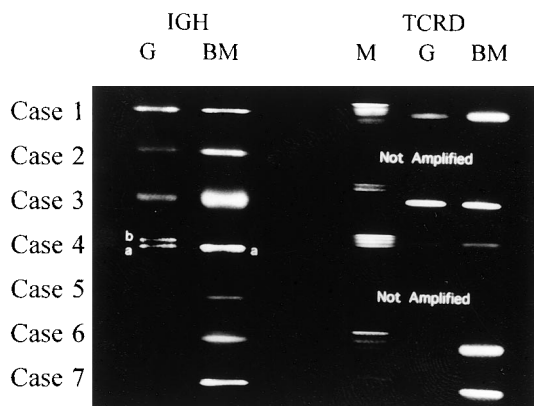


Figure 2. PCR analysis for rearranged IGH and TCRD. Amplification of rearranged IGH and TCRD from 7 cases in our study. Comparison of the PCR products from cases 1, 2, and 3 showed no size differences between Guthrie card and leukemic cell DNAs representing the IGH. We could not detect the rearranged TCRD from leukemic cell DNA and corresponding Guthrie card DNA in case 2. For case 4, the rearranged IGH product from Guthrie card DNA consisted of 2 different-sized fragments (4a and 4b), 1 of which (4a) was identical to the fragment amplified from leukemic cell DNA. Sequencing of the (a) and (b) DNAs revealed the same V-D-J or V-D rearrangements (Tables 3 and 4). M indicates DNA size marker; G, PCR products from Guthrie card DNA; and BM, PCR products from leukemic bone marrow cell DNA.

IGH rearrangements (4a and 4b) were amplified from Guthrie card DNA, but 1 of these bands, 4b, was not detectable in leukemic cells, even though the 4a sequences were identical. Overall, the N regions of IGH sequences consisted of 0 to 6 nucleotide insertions in 5 of the 7 cases. By contrast, the TCRD N regions possessed only 1 or 2 nucleotide insertions in the 5 cases analyzed, in which 3 cases had identical TCRD sequences in leukemic bone marrow cell and Guthrie card DNAs.

Discussion

Demonstrating the clonal origin of leukemic cells can be difficult. One accepted method is to trace specific karyotypic changes to a suspected precursor cell; however, this option is eliminated when Guthrie cards are the only source of prediagnostic genetic material. Moreover, as in the present study, it may not be possible to extract RNA from Guthrie card blood spots, obviating the use of reverse transcriptase-PCR to detect leukemia-specific chimeric gene expression.

Identification of antigen-receptor gene rearrangements affords an attractive alternative for backtracking the clonal evolution of leukemic cells. The IGH and TCRD regions, for example, undergo rearrangements that are unique for an individual cell and its progeny. Since these changes occur in 95% and 54% of the cases, respectively, we reasoned that a combination of these markers would allow amplification of rearranged gene segments in all but a small proportion of cases. Indeed, in recent studies, this strategy was successfully used to detect minimal residual disease in ALL patients who were receiving chemotherapy or had undergone bone marrow transplantation.²³⁻²⁷

Here we report identically rearranged IGH or TCRD segments in Guthrie card and leukemia cell DNA from 4 patients with ALL (ages 14 days, 2 months, 2.2 years, and 1.5 years). In case 4, we detected the biallelic rearranged IGH products (4a and 4b) from Guthrie card DNA. On the other hand, we amplified the single allele region (4a). This result suggested that 2 clonal populations may have existed at birth or that continuing rearrangement or the deletion of the allele may have occurred in the process of developing leukemia. However, the precise mechanism remains to be determined. In cases 5, 6, and 7, we attempted to demonstrate the clonal band by preparing allele-specific primers, which are more sensitive than the consensus primers, but no amplification was obtained with this procedure. Failure to demonstrate leukemic progenitor cells in the neonatal blood spots of 3 patients could indicate the absence of leukemia or too few leukemic cells for detection with our PCR methods. The probability that identical V-D-J or V-D sequences were detected by chance alone is vanishingly small—occurring in 1 in 10^6 to 1 in 10^8 cells under our study conditions. Thus, taken together, our data suggest the presence of leukemic progenitor cells in the neonatal blood spots in at least 4 of the 7 patients studied. The fetal characteristics of the leukemic cells were indicated by the small numbers of nucleotides inserted into the N region, the shortened D germ line and conserved J germ-line segments (IGH), and the conserved germ-line sequences of V_D2 and D_D3 (TCRD).²⁸⁻³⁰

It may be important that each of the patients with positive findings in Guthrie card blood spots developed overt leukemia within 2.5 years after birth. This result agrees with previous molecular studies demonstrating the presence of leukemic cells in neonatal blood specimens and suggests a relatively rapid onset of disease in patients with Guthrie card evidence of fetally transformed lymphoid cells. However, a recent account of identical twins who developed T-cell leukemia at 9 or 10 years of age provides a strong argument for protracted latency in isolated cases originating from fetal cells.

In infants, B-cell precursor ALL is characterized by hyperleukocytosis, the absence of surface CD10 expression, rearrangements of the *MLL* gene on chromosome 11q23, and a generally poor response to chemotherapy. By contrast, in children 2 to 4 years of age, the disease tends to be associated with a low presenting leukocyte count, positive CD10 expression, and good response to standard chemotherapy.³¹ Since both types of ALL originate, and probably begin clonal proliferation, during the fetal period, the reason for their dissimilar clinical features is unclear. Continued study of Guthrie card neonatal blood using immunologic markers to detect clonotypic changes should help answer this question and others regarding the natural history of childhood ALL.

Acknowledgment

The authors are grateful to Yasuko Hashimoto for her assistance in the preparation of this manuscript.

References

- McWhirter WR, Dobson C, Ring I. Childhood cancer incidence in Australia, 1982-1991. *Int J Cancer*. 1996;65:34-38.
- Pendergrass TW. Epidemiology of acute lymphoblastic leukemia. *Semin Oncol*. 1985;12:80-91.
- Inskip HM, Hall AJ, Temple IK, et al. Response to hepatitis B vaccine in relation to the hepatitis B status of family members. *Int J Epidemiol*. 1991; 20:770-773.
- Knox EG, Marshall T, Barling R. Leukaemia and childhood cancer in twins. *J Epidemiol Community Health*. 1984;38:12-16.
- Ford AM, Bennett CA, Price CM, Bruin MC, Van WE, Greaves M. Fetal origins of the TEL-AML1 fusion gene in identical twins with leukemia. *Proc Natl Acad Sci U S A*. 1998;95:4584-4588.
- Wiemels JL, Cazzaniga G, Daniotti M, et al. Prenatal origin of acute lymphoblastic leukaemia in children. *Lancet*. 1999;354:1499-1503.
- Gale KB, Ford AM, Repp R, et al. Backtracking leukemia to birth: identification of clonotypic gene

- fusion sequences in neonatal blood spots. *Proc Natl Acad Sci U S A*. 1997;94:13950-13954.
8. Bernard OA, Romana SP, Poirel H, Berger R, Molecular cytogenetics of t(12;21)(p13;q22). *Leuk Lymphoma*. 1996;23:459-465.
 9. Griesinger F, Greenberg JM, Kersey JH. T cell receptor gamma and delta rearrangements in hematologic malignancies: relationship to lymphoid differentiation. *J Clin Invest*. 1989;84:506-516.
 10. Biondi A, Francia di Celle P, Rossi V, et al. High prevalence of T-cell receptor V delta 2-(D)-D delta 3 or D delta 1/2-D delta 3 rearrangements in B-precursor acute lymphoblastic leukemias. *Blood*. 1990;75:1834-1840.
 11. Breit TM, Wolvers-Tettero IL, Beishuizen A, Verhoeven MA, van Wering ER, van Dongen JJ. Southern blot patterns, frequencies, and junctional diversity of T-cell receptor-delta gene rearrangements in acute lymphoblastic leukemia. *Blood*. 1993;82:3063-3074.
 12. Yokota S, Hansen HT, Ludwig WD, et al. Use of polymerase chain reactions to monitor minimal residual disease in acute lymphoblastic leukemia patients. *Blood*. 1991;77:331-339.
 13. Szczepanski T, Langerak AW, Wolvers TI, et al. Immunoglobulin and T cell receptor gene rearrangement patterns in acute lymphoblastic leukemia are less mature in adults than in children: implications for selection of PCR targets for detection of minimal residual disease. *Leukemia*. 1998;12:1081-1088.
 14. Felix CA, Poplack DG, Reaman GH, et al. Characterization of immunoglobulin and T-cell receptor gene patterns in B-cell precursor acute lymphoblastic leukemia of childhood. *J Clin Oncol*. 1990;8:431-442.
 15. Diaz MA, Garcia SF, Vicario JL, Ramirez M, Balas A, Madero L. Clinical relevance of T-cell receptor delta gene rearrangements in childhood B-precursor cell acute lymphoblastic leukaemia. *Br J Haematol*. 1997;99:308-313.
 16. Tonegawa S. Somatic generation of antibody diversity. *Nature*. 1983;302:575-581.
 17. Yamada M, Hudson S, Tournay O, et al. Detection of minimal disease in hematopoietic malignancies of the B-cell lineage by using third-complementarity-determining region (CDR-III)-specific probes. *Proc Natl Acad Sci U S A*. 1989;86:5123-5127.
 18. Potter MN, Steward CG, Maitland NJ, Oakhill A. Detection of clonality in childhood B-lineage acute lymphoblastic leukaemia by the polymerase chain reaction. *Leukemia*. 1992;6:289-294.
 19. Buluwela L, Albertson DG, Sherrington P, Rabbits PH, Spurr N, Rabbits TH. The use of chromosomal translocations to study human immunoglobulin gene organization: mapping DH segments within 35 kb of the C mu gene and identification of a new DH locus. *Embo J*. 1988;7:2003-2010.
 20. Ichihara Y, Matsuoka H, Kurosawa Y. Organization of human immunoglobulin heavy chain diversity gene loci. *Embo J*. 1988;7:4141-4150.
 21. Matsuda F, Shin EK, Hirabayashi Y, et al. Organization of variable region segments of the human immunoglobulin heavy chain: duplication of the D5 cluster within the locus and interchromosomal translocation of variable region segments. *Embo J*. 1990;9:2501-2506.
 22. Matsuda F, Shin EK, Nagaoka H, et al. Structure and physical map of 64 variable segments in the 3'0.8-megabase region of the human immunoglobulin heavy-chain locus. *Nat Genet*. 1993;3:88-94.
 23. Marshall GM, Kwan E, Haber M, et al. Characterization of clonal immunoglobulin heavy chain and I cell receptor gamma gene rearrangements during progression of childhood acute lymphoblastic leukemia. *Leukemia*. 1995;9:1847-1850.
 24. Wu NH, Lu SG, Zhu P, Peng YY. Detection of minimal residual disease in childhood acute lymphoblastic leukemia after termination of therapy. *Pediatr Hematol Oncol*. 1996;13:257-263.
 25. Beishuizen A, Verhoeven MA, van Wering ER, Hahlen K, Hooijkaas H, van Dongen JJ. Analysis of Ig and T-cell receptor genes in 40 childhood acute lymphoblastic leukemias at diagnosis and subsequent relapse: implications for the detection of minimal residual disease by polymerase chain reaction analysis. *Blood*. 1994;83:2238-2247.
 26. Steward CG, Goulden NJ, Katz F, et al. A polymerase chain reaction study of the stability of Ig heavy-chain and T-cell receptor delta gene rearrangements between presentation and relapse of childhood B-lineage acute lymphoblastic leukemia. *Blood*. 1994;83:1355-1362.
 27. Campana D, Pui CH. Detection of minimal residual disease in acute leukemia: methodologic advances and clinical significance. *Blood*. 1995;85:1416-1434.
 28. Bangs LA, Sanz IE, Teale JM. Comparison of D, JH, and junctional diversity in the fetal, adult, and aged B cell repertoires. *J Immunol*. 1991;146:1996-2004.
 29. Steenberg EJ, Verhagen OJ, van Leeuwen EF, et al. B precursor acute lymphoblastic leukemia third complementarity-determining regions predominantly represent an unbiased recombination repertoire: leukemic transformation frequently occurs in fetal life. *Eur J Immunol*. 1994;24:900-908.
 30. Wasserman R, Galili N, Ito Y, Reichard BA, Shane S, Rovera G. Predominance of fetal type DJH joining in young children with B precursor lymphoblastic leukemia as evidence for an in utero transforming event. *J Exp Med*. 1992;176:1577-1581.
 31. Pui CH, Kane JR, Crist WM. Biology and treatment of infant leukemias. *Leukemia*. 1995;9:762-769.