Different Molecular Consequences of the 1;19 Chromosomal Translocation in Childhood B-Cell Precursor Acute Lymphoblastic Leukemia

By Enrica Privitera, Mark P. Kamps, Yasuhide Hayashi, Toshiya Inaba, Linda H. Shapiro, Susana C. Raimondi, Frederic Behm, Linda Hendershot, Andrew J. Carroll, David Baltimore, and A. Thomas Look

The prognostically important 1;19 chromosomal translocation can alter the E2A gene on chromosome 19p13 in childhood B-cell precursor acute lymphoblastic leukemia (ALL), leading to formation of a fusion gene (E2A-PAHX) that encodes a hybrid transcription factor with oncogenic potential. It is not known whether this molecular alteration is a uniform consequence of the t(1;19) or is restricted to translocation events within specific immunologic subtypes of the disease. Therefore, we studied leukemic cells from 25 cases of B-cell precursor ALL, with or without evidence of cytoplasmic Ig heavy chains (clg); 17 cases had the t(1;19) by cytogenetic analysis. Leukemic cell DNA samples were analyzed by Southern blotting to detect alterations within the E2A genomic locus; a polymerase chain reaction assay was used to identify expression of chimeric E2A-PBX1 transcripts in leukemic cell RNA; and immunoblotting with anti-PBX1 antibodies was used to detect hybrid E2A-PBX1 proteins. Of 11 cases of clg' ALL with the t(1;19), 10 had E2A-PBX1 chimeric transcripts with identical junctions and a characteristic set of E2A-PBX1 hybrid proteins. Each of these cases had E2A gene rearrangements, including the one in which fusion transcripts were not detected. By contrast, none of the six cases of t(1;19)-positive, clg' ALL had evidence of rearranged E2A genomic restriction fragments, detectable E2A-PBX1 chimeric transcripts, or hybrid E2A-PBX1 proteins. Typical chimeric E2A-PBX1 transcripts and proteins were detected in one of eight clg' leukemias in which the t(1;19) was not identified by cytogenetic analysis, emphasizing the increased sensitivity of molecular analysis for detection of this abnormality. We conclude that the molecular breakpoints in cases of clg' B-cell precursor ALL with the t(1;19) differ from those in clg cases with this translocation. Leukemias that express hybrid oncoproteins such as E2A-PBX1 or Bcr-Ab1 have had a poor prognosis in most studies. Thus, molecular techniques to detect fusion genes and their aberrant products should allow more timely and appropriate treatment of these aggressive subtypes of the disease. © 1992 by The American Society of Hematology.

CHROMOSOMAL TRANSLocations appear to be important events in the development of the human leukemias. In acute lymphoblastic leukemia (ALL), more than 20 different nonrandom rearrangements have been identified. One of the most frequent is the t(1;19), which involves the long arm of chromosome 1 (at q23) and the short arm of chromosome 19 (at p13). This translocation is found in the leukemic cells of 6.5% of children with ALL, including about 25% of the cases with cytoplasmic Ig heavy chains (clg) in the lymphoblasts and 1% of clg' cases with markers of early B-cell progenitors. Molecular analysis of clg' cases with the t(1;19)(q23;p13) has demonstrated that the translocation breakpoint on chromosome 19 occurs within a single intron of the E2A gene, which encodes transcription factors containing a helix-loop-helix DNA binding motif and dimerization domain. E2A proteins were originally identified by their ability to bind to the κE2A DNA sequence motif contained in the Ig κ light-chain enhancer. Similarly, the breakpoint on chromosome 1q23 interrupts a homeobox gene known as PBX1 (formerly PRL). As a result, chimeric transcripts are formed that fuse coding sequences of the E2A gene in frame with PBX1 sequences. Chimeric E2A-PBX1 proteins are produced that retain the activator domain of the E2A protein but substitute a homeobox domain of the PbX1 protein for the helix-loop-helix DNA-binding and dimerization domain of E2A. At least two chimeric E2A-PBX1 transcripts are produced by alternative splicing in leukemic cells, resulting in a family of chimeric proteins that may function as aberrant transcription factors. When cDNAs encoding two of the hybrid E2A-PBX1 proteins were introduced into mouse NIH-3T3 fibroblasts, the resulting cells were tumorigenic in nude mice, indicating that these hybrid gene products might contribute to leukemic cell growth.

Recent studies show that clg' B-cell precursor ALL with the t(1;19) may respond poorly to treatment that is effective against other subtypes of B-lineage leukemia. If most clg' cases contain identical E2A-PBX1 chimeric transcripts and proteins, then molecular detection of these aberrant products would afford an attractive diagnostic alternative to conventional cytogenetic studies, which are difficult to perform in human leukemic cells and may yield apparently identical results for rearrangements that have completely different molecular consequences.

In this report, we demonstrate identical hybrid E2A-PBX1 transcripts and proteins in most cases of clg' ALL with the t(1;19), but could not detect such features in cytogenetically indistinguishable t(1;19) cases that were clg'. Thus, molec...

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ular approaches are definitive and efficient in detecting the clinically important subgroup of childhood leukemia patients with E2A-PBX1 rearrangements in their malignant cells.

**MATERIALS AND METHODS**

**Patients and lymphoblast samples.** This study included 25 patients who were admitted to St Jude Children’s Research Hospital or to institutions participating in the Pediatric Oncology Group. Leukemic cells were obtained at the time of diagnosis from bone marrow aspirates and cryopreserved (2 x 10^6 cells per milliliter of medium containing 10% fetal calf serum and 10% dimethylsulfoxide). There were 17 cases of B-cell precursor ALL with the t(1;19) (11 cIg⁺ and 6 cIg⁻), and eight cytogenetically characterized control cases that lacked the t(1;19) (Table 1). Written informed consent was obtained from the patients (when appropriate) and their parents or guardians, and the protocols for therapeutic and biologic studies were reviewed and approved by the institutions’ human investigations review boards.

**Immunophenotyping and cytogenetic analysis.** Bone marrow samples were studied for both the karyotype and the immunophenotype of the leukemic clones. Standard indirect immunofluorescence assays with monoclonal antibodies to lymphoid-associated antigens were used to classify cells as B-cell precursor (CD19⁺, CD22⁺, CD10*, HLA-DR⁺, CD7⁻, CD5⁻), T cells (CD7⁻, CD5⁻, CD8⁺ or CD2⁻), or mature B cells (surface Igs⁺). Leukemias with immunophenotypes of T cells (CD7⁻, CD5⁻) and without cIg expression. Leukemias with immunophenotypes of T cells (CD7⁻ plus CD5⁻ or CD2⁻) and mature B cells (surface Igs⁺) were excluded from further study. Leukemic cell karyotypes were determined by previously reported techniques.⁷ Our designation of B-cell precursor leukemias as cIg⁺ and cIg⁻ corresponds to the pre-B and early pre-B classifications used by the Pediatric Oncology Group.⁴

**Analysis of E2A gene rearrangements in blasts cell DNA.** High-molecular-weight DNA was extracted from leukemic lymphoblasts, digested with restriction endonucleases, subjected to electrophoresis in 0.8% agarose gels, and transferred to nylon membranes as previously described.⁸ Filters were hybridized under stringent conditions with a 3²P-labeled E2A cDNA probe (pE47M), containing a 300-bp fragment that spans the translocation junction site.¹¹ Several restriction enzymes, including EcoRI, BamHI, and HindIII, were used initially, but Bgl II and Xba I most often showed rearranged bands due to the translocation; hence, they were used in all further analyses of DNA.

**Detection of chimeric E2A-Pbx1 transcripts.** Using cDNA sequences representing the chimeric E2A-Pbx1 and normal E2A transcripts, we prepared a series of oligonucleotides for amplification of the RNA sequences by the polymerase chain reaction (PCR). The oligonucleotides included E5 (5' TGCACAAC-CAGCGGCCCTC 3'), E2A (5' CCCCTTCTCCCTCT- CGAGTTGT 3'), used as sense and antisense strand primers for normal E2A RNA, and P3 (5' CGCCACGCCTCGCTACACA 3'), a pbx1 antisense strand primer reverse complementary to chimeric sequences 90 bp from the translocation junction. Oligonucleotide E2A (5' CGAGTCTCACAGTTTTAGATACC 3'), which contained 13 bp on each side of the E2A-pbx1 junction site, was used as a specific probe to detect products amplified from chimeric transcripts on Southern blots. Similarly, oligonucleotide E2A (5' CGACTCTCACAGTGCTAGGCGAG 3') used as a probe for detection of cIg⁺ cases amplified from normal E2A transcripts. Total RNA was extracted from leukemic cells by the single-step method of Chomczynski and Sacchi.¹² cDNA was synthesized from 1 µg of total RNA with M-MLV reverse transcriptase (200 U) in PCR buffer (Perkin-Elmer Cetus, Norwalk, CT) containing random hexamer primers (100 pmol), deoxynucleotide triphosphates (1 mmol/L each), and RNasin (20 U) at 37°C for 1 hour (final reaction volume, 20 µL). The mixture was then denatured at 95°C for 5 minutes and cooled on ice. For amplification of the cDNA products, oligonucleotide primers were added at a final concentration of 0.5 µmol/L in a total volume of 100 µL. Taq polymerase (2.5 U; Perkin-Elmer Cetus) was then added and 40 cycles of amplification were performed in a DNA thermal cycler (Perkin-Elmer Cetus) with a stepwise program consisting of 95°C for 0.5 minutes, 62°C for 0.5 minutes, and 72°C for 0.5 minutes. The PCR products were separated by electrophoresis on 1.2% agarose gels, transferred to nylon membranes, and hybridized to either the E or EP radiolabeled oligonucleotide probes. RNAs from the 697 cIg⁺, B-cell precursor line [t(1;19)⁺], and the HL-60 myeloid cell line [t(1;19)⁻] were used as positive and negative controls, respectively. To avoid false-positive results in sensitive PCR assays that could be due to contamination of reaction mixtures with small amounts of the E2A-Pbx1 template, we used disposable glass microcapillary pipettes for RNA templates and positive displacement pipettes with disposable tips and plungers for other components. As an additional precaution, tubes containing buffers and oligonucleotides were treated with UV radiation (254 nm) for 5 minutes before they were added to the cDNA templates for PCR assays.

**Detection of E2A-Pbx1 and cIg proteins.** To identify E2A-Pbx1 proteins, leukemic cells were solubilized by boiling in sodium dodecyl sulfate (SDS)-containing Laemmli sample buffer at a concentration of 2 x 10⁶ cells per milliliter. Aliquots of these samples equivalent to 4 x 10⁶ cells were resolved by electrophoresis in SDS-denatured polyacrylamide gels and transferred to nitrocellulose. Filters were incubated with 10 µg/mL affinity-purified anti-PBX Ig followed by alkaline phosphatase-conjugated goat antirabbit Ig, as previously described.¹³ For detection of Ig μ heavy chains, 2 x 10⁶ cells were solubilized in 1 mL of NP40 lysis buffer (0.05 mol/L Tris HC1, pH 7.4, 150 mmol/L NaCl, 0.5%, NP40, and 0.5% sodium deoxycholate). Nuclei were pelleted and lysates were immunoprecipitated with goat antibodies to human Ig μ heavy chains, followed by protein A- sepharose. The samples were then resolved by electrophoresis under denaturing conditions in polyacrylamide gels containing SDS. After electrophoretic transfer of proteins to nitrocellulose, filters were incubated with goat antibodies specific for human Ig μ chains, followed by ¹²⁵I-labeled protein A.

**RESULTS**

**Immunophenotypic and cytogenetic features related to the t(1;19).** The 17 cases of t(1;19)⁺ B-cell precursor ALL that we studied are described in Table 1. Six cases had a balanced t(1;19) with retention of both derivative chromosomes, and 11 had only the derivative 19 chromosome, with loss of the derivative 1. The derivative chromosomes produced by the t(1;19) were indistinguishable by microscopic analysis of Giemsa-banded chromosomes, whether they were found in cIg⁺ or cIg⁻ cases. Leukemic cell karyotypes of the 11 cIg⁺ cases often exhibited chromosomal rearrangements in addition to the t(1;19) and were generally in the near-diploid range (46 to 49 chromosomes). By contrast, all but one of the six cIg⁻ cases were hyperdiploid with greater than 50 chromosomes per leukemic cell, owing to trisomies primarily involving chromosomes X, 4, 6, 10, 14, 18, and 21. Patients with the t(1;19) in their leukemic lymphoblasts showed no obvious differences in gender, age, or leukocyte count at diagnosis whether the blasts were classified as cIg⁺ or cIg⁻ (Table 1).
Table 1. Diagnostic Chromosomal and Molecular Genetic Findings in the Lymphoblasts of Patients With B-Cell Precursor ALL

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Leukocyte Count (x10⁹/L)</th>
<th>Karyotype*</th>
<th>DNA Rearrangement by Southern Blot Analysis</th>
<th>E2A-pbx1 Transcripts by PCR Analysis of RNA</th>
<th>E2A-Pbx1 Protein by Immunoblot Analysis†</th>
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<tbody>
<tr>
<td>t(1;19)+, clg'</td>
<td>1</td>
<td>F</td>
<td>5.0</td>
<td>106.0</td>
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<td>+</td>
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<td>+</td>
</tr>
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<td>4</td>
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<td>10.5</td>
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<td></td>
<td>5</td>
<td>M</td>
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<td>16.3</td>
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<td>ND</td>
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<td>M</td>
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<td>11</td>
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<td>3.2</td>
<td>25.7</td>
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<td>25</td>
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<td>t(1;19)+, clg'</td>
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<td>M</td>
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<td>18.2</td>
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<tr>
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<td>15</td>
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<td>3</td>
<td>6.9</td>
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<td>ND</td>
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<tr>
<td></td>
<td>17</td>
<td>M</td>
<td>7</td>
<td>11.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

(Continued on following page)

E2A gene rearrangements. Using Southern blot analysis and an E2A CDNA probe, we studied leukemic cell DNA from 13 of the 17 cases of t(1;19)+ ALL, as well as cases lacking this translocation. Rearranged restriction fragments were detected in DNA from each of the tested clg' leukemias containing the t(1;19), as illustrated by the four representative cases shown in Fig 1. Interestingly, rearranged fragments were not detected in the three cases of early B-cell precursor leukemia with the t(1;19) whose patterns of normal E2A restriction fragments resembled those of leukemias without this translocation (Fig 1).

E2A-pbx1 chimeric transcripts. Previous reports of analysis of clg' leukemias suggest that the chromosomal breakpoints of the t(1;19) affect the same introns in every
instance, resulting in expression of identical chimeric 
RNAs. Therefore, we prepared oligonucleotides 
specific for sequences on each side of the breakpoint and used 
them to amplify cDNA templates synthesized from leuke-
mic cell RNA (Fig 2A). With a radiolabeled oligonucle-
otide probe specific for sequences spanning the E2A-pbx1 
junction, we detected transcripts in seven of eight cIg+ 
cases (patient 11, lane 1, Fig 3), in which the leukemic cells 
share antigenic determinants with Pbx1. None of the five fusion proteins were present in the five 
cIg- leukemic cells lacking the t(1;19) (patient 24, lane 25, 
and Table 1), whose cIg- lymphoblasts had a t(1;19) but no detectable E2A-pbx1 transcripts 
(lane 30, Fig 2B).

E2A-pbx1 expression was also detected in leukemic 
lymphoblasts from one of the eight patients with cIg+ 
leukemia and no t(1;19) (patient 24, Fig 2B and 
Table 1). This child’s leukemic cell karyotype differed from the 
others in that only normal chromosome carriers, and that leukemic metaphases, presumably 
containing a t(1;19), were not identified because of low 
leukemic cell mitotic indices.

Chimeric E2A-Pbx1 proteins. Because E2A-Pbx1 fusion 
proteins are produced as a result of the t(1;19), and Pbx1 proteins are not normally expressed in the early stages of 
B-cell differentiation,11 we analyzed leukemic cells by immu-
noblot analysis with affinity-purified anti-Pbx1 Igs. In 10 of the 
11 cases of cIg+ ALL with the t(1;19), the blast cells expressed a characteristic array of E2A-Pbx1 proteins, as 
illustrated in Fig 3. These hybrid proteins have been shown to result, at least in part, from differential exon usage within the Pbx1 gene.12 Two less abundant proteins were stained 
by anti-Pbx1 antibodies in one of the t(1;19)-containing cIg+ 
cases (patient 11, lane 1, Fig 3), in which the leukemic cells 
had rearranged genomic E2A, as detected by Southern blot 
analysis (data not shown), but lacked the fused E2A-pbx1 
RNA (Fig 2B). Although these proteins migrated closely 
with two of the five characteristic E2A-Pbx1 chimeric 
proteins, their weak reactivity with anti-Pbx1 Ig is also 
consistent with the possibility that they represent the 
products of a fusion between E2A and a Pbx1-related gene. In 
addition to E2A-Pbx1 proteins in cIg+, t(1;19) cases, the 
affinity-purified rabbit anti-Pbx1 antibodies stained approx-
imately 20 polypeptides in all samples. Antibodies from a 
second rabbit that were also affinity-purified against recom-
binant Pbx1 protein stained the same polypeptides, indicat-
ing that these cross-reactive polypeptides are either the 
products of Pbx1-related genes or unrelated proteins that 
share antigenic determinants with Pbx1.

None of the five fusion proteins were present in the five 
cases of cIg- ALL with the t(1;19) (Fig 3), consistent with 
PCR results showing an absence of E2A-pbx1 RNA (Fig 
2B). The only cIg- leukemic cells lacking the t(1;19) that 
showed the typical set of five hybrid E2A-Pbx1 proteins 
were those from patient 24, underscoring the close correla-
tion between PCR and immunoblot assays in detecting 
these products.

cIg analysis. Cytoplasmic Ig μ heavy chains were analyzed in each case by a sensitive immunofluorescence slide 
assay that distinguishes intracellular from plasma mem-
brane expression of these molecules. Because molecular 
studies suggested that the cIg- cases did not have rearrange-
ments of the E2A gene or express E2A-pbx1 RNA or

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Table 1. Diagnostic Chromosomal and Molecular Genetic Findings in the Lymphoblasts of Patients With B-Cell Precursor ALL (Cont'd)

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Leukocyte Count (x 10⁹/L)</th>
<th>Karyotype*</th>
<th>DNA Rearrangement by Southern Blot Analysis</th>
<th>E2A-pbx1 Transcript by PCR Analysis of RNA</th>
<th>E2A-Pbx1 Protein by Immunoblot Analysis†</th>
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<tbody>
<tr>
<td>t(1;19)−cIg−</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>18</td>
<td>M</td>
<td>5.1</td>
<td>23.8</td>
<td>46,XY,dup(1)(q11→q44)/46,XY,tan dup(1)(q11→q44)</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>19</td>
<td>M</td>
<td>2.1</td>
<td>124.0</td>
<td>46,XY,del(8)(q19q21)</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>20</td>
<td>F</td>
<td>1.7</td>
<td>164.0</td>
<td>46,XX,t(4;11)(q21;q23)</td>
<td>–</td>
<td>ND</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>F</td>
<td>4.8</td>
<td>38.2</td>
<td>46,X→X,+del(X)(p21)</td>
<td>ND</td>
<td>–</td>
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<tr>
<td>22</td>
<td>F</td>
<td>4.0</td>
<td>5.0</td>
<td>45,X→X,+der(X)(t(X;7)(p22;7),13,del(8)(p22),del(12)(p12:p13)/45,X→X,+der(X)(t(X;7),13,del(8)</td>
<td>–</td>
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<td>–</td>
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<tr>
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<td>M</td>
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<td>50.5</td>
<td>47,XY,X+X/47,XY,X,+t(11;?)</td>
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<td>–</td>
<td>–</td>
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<td>63.2</td>
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<td>12.5</td>
<td>3.1</td>
<td>45,XX,del(14)(q22q31)/46,XX,i(9q),i(17q)</td>
<td>ND</td>
<td>–</td>
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</table>

Abbreviation: tan dup, tandem duplication.

*The leukemic cell karyotypes have been previously reported, except for those of patients 7, 8, 13-17, 23.
†The immunoblot results are shown in Fig 3 for patients 3, 5, 6, 7, 11, 13-19, and 22-25. Results were previously reported for patients 1, 2, 4, 8, and 9.13
‡Constitutional cytogenetic abnormality.
§Only two proteins were identified by immunoblot analysis in lysates of this patient’s leukemic cells, in contrast to the 5 characteristic E2A-Pbx1 proteins found in the other positive leukemias.
‖This karyotype is likely to represent residual normal marrow cells (see text).
MOLECULAR HETEROGENEITY OF THE t(1;19) IN ALL

Expression of the E2A-PBX1 fusion gene, resulting from the 1;19 chromosomal translocation in cases of clg⁺ ALL, was reliably detected by molecular analysis demonstrating E2A gene rearrangements, chimeric E2A-pbx1 transcripts, and hybrid E2A-Pbx1 proteins in leukemic cells. These chimeric gene products were not found in clg⁻ cases that harbored the t(1;19), even though the chromosomal breakpoints were indistinguishable by analysis of Giemsa-banded chromosomes. Our results suggest that neither the E2A nor the PBX1 genes are altered by the translocations found in clg⁺ cases, indicating the need for analysis of other genes that might be affected by the t(1;19) chromosomal breakpoints in clg⁺ B-cell precursor ALL. Candidate genes located on the short arm of chromosome 19 include the LYL-1 gene, which encodes a helix-loop-helix protein with similarities to E2A, and the 4V cellular proto-oncogene.

The combination of molecular strategies used to identify characteristic E2A-PBX1 rearrangements has several advantages. For example, the leukemic cells from patient 11 lacked E2A-pbx1 RNAs in PCR assays, as well as the typical set of hybrid proteins found in other cases, despite evidence of rearrangements within the E2A gene by Southern blot analysis. Bands suggestive of two Pbx fusion proteins were identified by immunoblot analysis. Together, these results support two possible interpretations. First, this patient’s leukemic cells may have contained an alternative junction between E2A and PBX1, resulting in a fusion transcript that excludes sequences recognized by the oligonucleotide primers used in the PCR assay. Such alternative breakpoints have been reported for BCR-ABL rearrangements in chronic myelogenous leukemia and Philadelphia chromosome-positive ALL.

Alternative, a PBX gene cluster may reside on chromosome 1, band q23, raising the possibility of fusions between E2A and other PBX genes. We have sequenced a second gene contained in the PBX subfamily of homeoboxes that is 88% identical to Pbx1 at the amino acid level (M.P.K., unpublished observation, February 1991). At this point we have no strong evidence that favors either of these possibilities. Hunger et al. also observed a rare clg⁺ case that lacked chimeric E2A-pbx1 transcripts, but they did not include analysis of hybrid proteins, so we cannot be certain if their patient was similar to ours (patient 11).

The E2A-Pbx1 fusion proteins produced in clg⁺ B-cell precursors with typical t(1;19) translocations are likely to play a critical role in pathogenesis. Previous studies have demonstrated conclusively that two of the five hybrid proteins (p85E2A-Pbx1 and p77E2A-Pbx1) result from alternatively spliced E2A-pbx1 mRNAs, which encode different carboxy-terminal amino acids. CDNA clones encoding these proteins were found to have different biologic activity when introduced into mouse fibroblasts: p77E2A-Pbx1 induced large transformed foci in fibroblast

DISCUSSION

Genetic alterations in developing B-lineage cells, affecting proteins involved in positive or negative regulation of cell growth and differentiation, can give rise to B-cell precursor ALL. In general, this subtype of leukemia responds well to chemotherapy and has a high likelihood of prolonged remission and cure. One of several exceptions appears to be clg⁺ ALL with the t(1;19), which has been related to a significantly worse clinical outcome than is usually seen in clg⁺ cases without this rearrangement or in most other subtypes of B-lineage ALL. Results of recent clinical trial suggest that the adverse prognostic impact of the t(1;19) in clg⁺ leukemias can be nullified with use of intensified chemotherapy. Thus, accurate early detection of the t(1;19) in cases of clg⁺ ALL would be important in selecting appropriate treatment. Interestingly, the t(1;19) found in clg⁺ B-cell precursor leukemias appears to signify a standard risk of relapse on less intensive therapy (ref 6 and our unpublished observations, July 1991). This better prognosis may be related to the absence of E2A-pbx1 transcripts and proteins, as opposed to those cases with a clg⁺ phenotype.

Fig 1. Southern blot analysis of leukemic cell DNA. Rearranged Bgl II restriction fragments (arrowheads) hybridizing to an E2A cDNA probe are evident only in DNA from lymphoblasts with the t(1;19), which also express cytoplasmic Ig (lanes 1 through 4). Normal E2A bands of 11 and 24 kb (asterisks) are evident in all of the DNA samples. The migration of Hindlll fragments of known sizes (in kilobases) from bacteriophage λ DNA is indicated at the left margin.

proteins, we further evaluated a subset of the cases for μ heavy chain expression by Western blot analysis. The results of Western blotting (Fig 4) agreed with those of the immunofluorescence slide assay, confirming that the expression of E2A-pbx1 chimeric transcripts and proteins was restricted to t(1;19)⁺ leukemias that express clg.

The combination of molecular strategies used to identify characteristic E2A-PBX1 rearrangements has several advantages. For example, the leukemic cells from patient 11 lacked E2A-pbx1 RNAs in PCR assays, as well as the typical set of hybrid proteins found in other cases, despite evidence of rearrangements within the E2A gene by Southern blot analysis. Bands suggestive of two Pbx fusion proteins were identified by immunoblot analysis. Together, these results support two possible interpretations. First, this patient’s leukemic cells may have contained an alternative junction between E2A and PBX1, resulting in a fusion transcript that excludes sequences recognized by the oligonucleotide primers used in the PCR assay. Such alternative breakpoints have been reported for BCR-ABL rearrangements in chronic myelogenous leukemia and Philadelphia chromosome-positive ALL.

Alternative, a PBX gene cluster may reside on chromosome 1, band q23, raising the possibility of fusions between E2A and other PBX genes. We have sequenced a second gene contained in the PBX subfamily of homeoboxes that is 88% identical to Pbx1 at the amino acid level (M.P.K., unpublished observation, February 1991). At this point we have no strong evidence that favors either of these possibilities. Hunger et al. also observed a rare clg⁺ case that lacked chimeric E2A-pbx1 transcripts, but they did not include analysis of hybrid proteins, so we cannot be certain if their patient was similar to ours (patient 11).

The E2A-Pbx1 fusion proteins produced in clg⁺ B-cell precursors with typical t(1;19) translocations are likely to play a critical role in pathogenesis. Previous studies have demonstrated conclusively that two of the five hybrid proteins (p85E2A-Pbx1 and p77E2A-Pbx1) result from alternatively spliced E2A-pbx1 mRNAs, which encode different carboxy-terminal amino acids. CDNA clones encoding these proteins were found to have different biologic activity when introduced into mouse fibroblasts: p77E2A-Pbx1 induced large transformed foci in fibroblast
Fig 2. Detection of E2A-pbx1 transcripts. Schematic representations of chimeric E2A-pbx1 and normal E2A transcripts are shown in (A), together with the PCR products obtained after amplification using the indicated oligonucleotide pairs. A 164-bp fragment of the E2A-pbx1 fusion transcript, spanning the junction site, was amplified with primers E5 and P3. A 180-bp fragment of the normal E2A transcript was also amplified as a control for RNA integrity, using the primers E5 and E3. The end-labeled oligonucleotide probes EP and E were used to detect the chimeric and the normal transcripts in Southern blot analysis of RNA PCR products (B). The E2A-pbx1 fragment was detected in RNA from t(1;19)+ lymphoblasts that also express cytoplasmic Ig (lanes 2 through 8), and in one clg' case (patient 24, lane 29) in which the t(1;19) was not detected by cytogenetic analysis. RNA from the 697 [t(1;19)+] and HL-60 [t(1;19)-] leukemic cell lines were tested as positive and negative controls, and reactions were performed in each experiment without RNA template (blank). Normal E2A transcripts were detected in all of the samples, indicating that each RNA was intact and free of inhibitors of the PCR reactions. The migration of Hae III fragments of known sizes (in kilobases) from bacteriophage φx174 DNA is indicated at the right margin.

Fig 3. Analysis of E2A-Pbx1 proteins. Anti-Pbx1 IgGs were used in immunoblot analysis to detect a set of characteristic E2A-Pbx1 chimeric proteins in lysates of all but one of the t(1;19)+, clg' leukemias (lanes 2 through 5), and in one clg' leukemia in which the t(1;19) was not detected (lane 11). Two bands appearing as a doublet (arrowhead), the lower of which is visible in the photograph, were detected in lysates from one of the clg' cases with the t(1;19) (lane 1).
MOLECULAR HETEROGENEITY OF THE t(1;19) IN ALL

FIG 4. Ig μ heavy chains detected by Western blotting. The 80-Kd Ig μ heavy chain is observed only in lysates from lymphoblasts with detectable cytoplasmic staining of μ heavy chains in immunofluorescence slide assays (clg+; lanes 1 through 5, 8, 9). B-cell precursors with the t(1;19) that failed to stain for clg in the slide assay (clg−) also lacked detectable μ chains by Western blotting (lanes 6 and 7). The migration of protein standards of known molecular weight (in kilodaltons) is indicated at the left margin.

monolayers, and p77E47−Pbx1 was more efficient in inducing both density- and anchorage-independent cell growth. Presumably, these proteins contribute to the transformation of B-cell precursors through the aberrant transcriptional activation of Pbx1-responsive genes. Pbx1 is not normally expressed at detectable levels by hematopoietic cells, including those of the B lineage, and one consequence of the fused gene is aberrant expression of this protein driven from the E2A gene promoter. In addition, the transactivating domain of E2A is likely to contribute functionally to the chimeric protein, as demonstrated experimentally by its activity in constructs in which E2A residues were joined to a Gal4 DNA-binding domain. Characterization of the sequences recognized by the Pbx1 DNA binding domain and the effects of E2A-PBX1 fusion genes on early B-cell growth and development should provide additional insights into the role of this rearrangement in leukemogenesis.

Only normal metaphases were found in case 24, a result that characterizes the cytogenetic analysis of 10% to 30% of cases of childhood ALL.6,20 Because normal metaphases in standard karyotypes generally represent residual normal marrow elements, direct detection of RNA transcripts or proteins is warranted to ensure uniform detection of leukemic cells harboring E2A-pbx1 rearrangements in clinical settings.

Studies of cell surface antigens and Ig gene rearrangements indicate that leukemic B-cell precursors are the counterparts of normal early B-lineage cells. This has led to the practice of classifying B-lineage leukemia according to immunologic findings, with as many as four antigenically distinct compartments recognized by some investigators.26 Results of the present study, together with reports of high-risk leukemias with chimeric BCR-ABL fusion transcripts and proteins,14,15 suggest that systematic characterization of molecular defects within the broad category of B-cell precursor leukemias would provide sharper (and perhaps more prognostically relevant) distinctions than have immunologic approaches.

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Different molecular consequences of the 1;19 chromosomal translocation in childhood B-cell precursor acute lymphoblastic leukemia

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