Acute Leukemias Associated With the 4;11 Chromosome Translocation Have Rearranged Immunoglobulin Heavy Chain Genes

By William M. Crist, Michael L. Cleary, Carlo E. Grossi, Edgar F. Prasthofer, Glen D. Heggie, George A. Omura, Andrew J. Carroll, Michael P. Link, and Jeffrey Sklar

Acute leukemia with the 4;11 chromosomal translocation occurs most frequently in infants and young adults and has a poor prognosis. Hyperleukocytosis and splenomegaly are frequently present. Most do not bear the common acute lymphocytic leukemia antigen or T antigens. Furthermore, they did not have sheep erythrocyte receptors nor did they express surface or cytoplasmic immunoglobulin or B cell precursor determinants. Electron microscopy analysis showed that blasts from patient 1 exhibited numerous monoribosomes, polyribosomes, and isolated strands of rough endoplasmic reticulum in their cytoplasm. These ultrastructural features are characteristic for both common acute lymphocytic leukemia and pre-B-ALL cells, but not for T-ALL or acute myelogenous leukemia cells. Peroxidase was undetectable in cells from both patients. Our study suggests that this disorder represents a unique subtype of leukemia. The cell of origin may be an early B cell progenitor that shares certain surface antigens with myeloid cells or a stem cell with the potential for both lymphoid and myelomonocytic differentiation.

MATERIALS AND METHODS

Both patients were studied at the University of Alabama in Birmingham hospitals between November 1980 and July 1984. All studies, except gene rearrangement analysis, were performed on fresh bone marrow cells from patient 1, whereas only cytogenetic studies, routine morphology, and cytochemistry (PAS, myeloperoxidase, Sudan Black, nonspecific esterase, and α-naphthyl esterase) were performed on samples from both patients. Our study suggests that this disorder represents a unique subtype of leukemia. The cell of origin may be an early B cell progenitor that shares certain surface antigens with myeloid cells or a stem cell with the potential for both lymphoid and myelomonocytic differentiation.
were performed on fresh bone marrow cells from patient 2. Other studies were carried out on cryopreserved blast cells from both patients. Patient 1 had >90% blast cells in his bone marrow and blood at the time of diagnosis and at the time when studies were performed. Patient 2 had >80% blasts in the bone marrow at the time of diagnosis and at relapse.

Cytogenetic studies. Bone marrow aspirates obtained at the time of diagnosis in patient 1 and both at the time of diagnosis and relapse in patient 2 were cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS) for 24 hours at 34 °C and were then exposed to colcemid (0.06 µg/mL) for 3.5 hours at 4 °C. Routine methods were used for culture harvest, slide preparation, and GTG-banding. In addition, direct chromosome preparations were examined.

Morphology and cytochemistry. Bone marrow aspirates were stained with Wright-Giemsa stain, PAS, Sudan Black, peroxidase, acid phosphatase, and chloroacetate esterase and o-naphthyl acetate esterase by standard techniques.

Gene rearrangement studies. High mol wt DNA from the leukemic blasts of each case was digested with appropriate restriction enzymes, and the products were electrophoresed in a 0.8% agarose gel. DNA fragments separated by electrophoresis were transferred out of the gels onto activated nylon membranes (Plasco, Woburn, Mass), as described by Southern. Filters were hybridized with nick-translated [32P]-radiolabeled pBR322 plasmid DNA carrying human immunoglobulin gene DNA fragments. The precise locations of these fragments within chromosomal DNA in the region of the immunoglobulin genes have been previously published. The DNA fragment used as a hybridization probe for heavy chain gene rearrangements is specific for the joining region of the heavy chain gene and detects heavy chain gene rearrangements regardless of the expressed heavy chain class. The C, and mixed C light chain

Terminal deoxynucleotidyl transferase activity.
Ig GENE REARRANGEMENT IN LEUKEMIA WITH t(4;11)

Table 1. Clinical Characteristics at Diagnosis, Response to Therapy, and Cytogenetic Studies

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Sex</th>
<th>Length of First Remission (mo)</th>
<th>WBCs ($\times 10^3$ L$^{-1}$)</th>
<th>Survival (mo)</th>
<th>Time of Study</th>
<th>No. of Cells Examined</th>
<th>Banded Karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22 yr/M</td>
<td>0</td>
<td>30.0</td>
<td>5.5</td>
<td>Diagnosis</td>
<td>16</td>
<td>4, 12</td>
</tr>
<tr>
<td>2</td>
<td>5.5 mo/F</td>
<td>18</td>
<td>4.1</td>
<td>24</td>
<td>Diagnosis</td>
<td>16</td>
<td>14, 2</td>
</tr>
</tbody>
</table>

Ultrastructural studies. Bone marrow cells from patient 1 consisted of $>90\%$ peroxidase-negative blasts. Nuclei were round in shape, with finely dispersed chromatin and numerous small nucleoli. Nuclear blebs were occasionally seen. The cytoplasm of all blasts contained a few mitochondria and was largely occupied by ribosomes, which were occasionally clustered to form polyribosomes. A few isolated strands of rough endoplasmic reticulum were also detected. A small Golgi apparatus was rarely seen, and electron-dense granules were absent (Fig 1). These ultrastructural features, including a well-developed ribosomal apparatus, a small Golgi complex, and scarce or absent lysosomes are usually observed in common or pre-B-ALLs, but not in T-ALLs or AMLs.25

Immunologic markers. Immunologic marker data are summarized in Table 2. The leukemic cells obtained at diagnosis from both patients expressed HLA-DR antigens and certain myelomonocytic antigens but failed to form ESR or express clg, slg, or T cell antigens. The blasts of neither patient expressed CALLA. All marker studies performed (see Materials and Methods), but not having results shown in Table 2, were negative, including the B cell-restricted antigens B1 and B4 (patient 1).

Gene rearrangement studies. Studies of the arrangement of immunoglobulin genes in DNA from the blast cells obtained from each patient at the time of diagnosis revealed that both heavy chain gene alleles were rearranged in each patient’s leukemic cells. The germline bands seen in the heavy chain analyses are presumably caused by residual

Fig 1. Ultrastructural localization of peroxidase activity in bone marrow cells from patient 1. Myeloid cells at various stages of maturation display numerous peroxidase-positive granules in the cytoplasm, and lymphoblasts are peroxidase negative. The insert illustrates some morphological features of a lymphoblast showing dispersed nuclear chromatin and a large nucleolus. The cytoplasm contains numerous ribosomes, single or in clusters, and isolated strands of rough endoplasmic reticulum. Original magnification $\times 4,000$, current magnification $\times 2,200$; insert, original magnification $\times 14,000$, current magnification $\times 7,700$. 

From www.bloodjournal.org by guest on August 30, 2017. For personal use only.
with non-T cell ALL. They found that tumor cells
antigens and, in some cases, the state of the
CALLA, T, clg, B), and the finding of rearranged
genes were present without light chain gene rearrangements.
These blast cells also expressed HLA-DR antigens but not
containing the 4;11 chromosome translocation and found
the earliest identifiable B progenitor cell.

We have studied the arrangement of the immunoglobulin
genes in the blast cells of two patients with acute leukemia
containing the 4;11 chromosome translocation and found
that in both cases rearrangements of both allelic heavy chain
genes were present without light chain gene rearrangements.
These blast cells also expressed HLA-DR antigens but not
CALLA or clg. EM studies revealed that cells from patient I
had ultrastructural features similar to those of c-ALL and
pre-B-ALL blasts. These findings support the viewpoint that
the cell of origin of these leukemias is an early B cell
genotyper. The immunologic phenotype (HLA-DR‘,
CALLA‘, T‘, clg‘, B‘), and the finding of rearranged
immunoglobulin heavy chain genes but not light chain genes
in the blasts of our patients, are similar to the features of
blast cells from patients with acute leukemia reported by
Korsmeyer et al. They proposed that such blasts represent
the earliest identifiable B progenitor cell.

Nadler et al7 have examined B cell-associated and B
cell-restricted antigens and, in some cases, the state of the
immunoglobulin genes of tumor cells from 138 children and
adults with non-T cell ALL. They found that tumor cells
from the patients could be assigned to one of four subgroups
based on patterns of antigen expression: HLA-DR Ia-like
antigen (Ia) alone (stage I); Ia/B4 (stage II); Ia/B4/
CALLA (stage III); and Ia/B4/CALLA/B1 (stage IV).
The expression of B cell-restricted antigens (B4 and B1) and
rearrangements of Ig heavy chain genes provided strong
evidence for the B cell lineage of stages II, III, and IV
tumors. They also found normal counterparts for tumor cells
of stages II through IV among fetal and adult bone marrow
cells. They concluded that the 4% of their patients who had
blasts that expressed only HLA-DR antigen (stage I) had
leukemias of uncertain lineage, but they speculated that the
blasts from this group represented an even earlier stage of
pre-B differentiation than did the IaB4-positive subgroup
(stage II). None of the stage I patients expressed Cµ. Results of
analyses of immunoglobulin gene rearrangements were
not reported for this group, and chromosomal studies were
not described for any of the cases in this study.

Our patients' leukemic cells also expressed certain myelomonocytic
antigens including 2D1, 4B3, 1C2, and/or Leu-M1. These antigens are expressed on myelomonocytic cells of
varying stages of maturation from the myeloblast to the
mature neutrophil, but not on T or B cells. Their reactivity
with early WBC progenitors is unknown. The expression of
myelomonocytic antigens on leukemic cells from patients of
similar phenotype studied by Korsmeyer et al and Nadler et
al was not reported.76,77

Recently, heavy chain gene rearrangements have been
shown to be not restricted solely to cells of B lineage.
Occasional examples of T cell ALL, Sezary cell syndrome,
AML, and a number of T cell lymphomas have demonstrated
heavy-chain gene rearrangements.26-33 It is possible that
the DNA rearrangements reported in these neoplasms do not
represent recombination of DNA for the three heavy chain
regions, variable (V), diversity (D), and joining (J).
Although bona fide D-J recombination has been well charac-
terized in T cell neoplasia, no example of V region recom-
bination has been described in cells other than B cells. It may
be that the latter event is restricted exclusively to B lineage
cells; however, our data cannot discriminate between D-J
and V-D-J recombination. New probes hybridizing with
DNA regions flanking the heavy chain immunoglobulin
genes will be required to resolve this issue. Nevertheless, it is
apparent that heavy chain Ig-gene rearrangements alone are
presently insufficient for assignment of B lineage and addi-
tional corroborating evidence is necessary.

Parkin et al7 have studied ten patients with acute leukemia
with the 4;11 translocation, using morphological, ultrastruc-

<table>
<thead>
<tr>
<th>Table 2. Laboratory Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphoid Markers*</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Patient No.</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>E Rosette</td>
</tr>
<tr>
<td>4/37°C</td>
</tr>
</tbody>
</table>

*Percentage of reactive cells by IFA determination.

Fig 2. Analyses of immunoglobulin gene configurations in
acute leukemias associated with the 4;11 translocation. DNA
isolated from cryopreserved bone marrow cells was analyzed for
immunoglobulin gene rearrangements by means of the Southern
blot hybridization procedure. For heavy chain (JH) and light chain
(Cλ) gene analysis, the DNA was cleaved with the restriction
endonuclease EcoRI. For a light chain gene analysis (Cµ) the DNA
was cleaved with the BamHI restriction enzyme. Under our hybrid-
ization conditions, a faint cross-hybridizing band of unknown
significance was detected with the Jµ probe as noted in lane 1 and as
we have previously published.9 Arrows denote the positions of
rearranged immunoglobulin bands: lines indicate the position of
germine bands: 1, patient 1 bone marrow cells; 2, patient 2 bone
marrow cells.
tural, cytochemical, and immunologic techniques. They concluded that blast cells from such patients may represent a proliferation of an early myeloid progenitor. They noted that the characteristic phenotype of these patients was ESR-2, s1g*, CALLA-*, Ba-1*, BA-2*, but HLA-DR* and ‘TdT*’. Six of these patients who were studied for s1g expression lacked it. The blasts from some of their patients had mast cell granules; some looked like monocytes and expressed nonspecific esterase. None of their patients had blasts that expressed myeloperoxidase or were Sudan Black B positive. Our patients had a similar phenotype, but their blasts were also shown to have immunoglobulin heavy chain gene rearrangements and to express certain myelomonocytic antigens. Morphologically, their leukemic cells were typically lymphoblastic (ie, FAB L1), and electron microscopy of blasts from patient 1 revealed ultrastructural features typical of an early B progenitor cell, including round nuclei with finely dispersed chromatin and numerous small nucleoli and abundant cytoplasmic ribosomes.29 Because cytochemical stains were negative in blasts from both of our cases, they failed to help establish the cell lineage.

Most investigators have concluded that the blasts of these patients are lymphoid cells based primarily on their lymphoid appearance. Most of these patients have responded to conventional therapy for acute lymphocytic leukemia, but their responses have been short and the overall prognosis is very poor.3,15 Our patient 1 failed to enter remission after receiving therapy appropriate for ALL or AML, whereas patient 2 entered remission with Vcr and Pdn after four weeks and remained in remission for 15 months on 6-mercaptopurine and methotrexate continuation therapy. The infant or young adult age of presentation, illustrated by our patients, has been frequently noted in other affected subjects.13 Hyperleukocytosis was noted at the time of diagnosis only in our patient 1.

We conclude that the immunoglobulin heavy chain gene rearrangements found in the DNA of blast cells from our patients, taken together with the findings of lymphoid morphology by light and electron microscopy (patient 1) and the expression of HLA-DR antigens and TdT (patient 1), but not CALLA, suggest that the cell of origin of the acute leukemia accompanied by the 4;11 translocation is an early B cell progenitor. The expression of certain myelomonocytic antigens is unexplained but may reflect: (1) a lack of specificity of these antigens, (2) their presence on early B cell progenitors, or (3) the potential for transformed B cell progenitors to express these antigens. In this regard, certain mouse monoclonal antibodies recognizing B cell precursors also detect a minority of peroxidase-positive myeloid cells.34 Alternatively, it is possible that the cell of origin of these leukemias is a common progenitor for B cells and myelomonocytic cells.

REFERENCES

19. Reinherz EL, King PC, Pesando JM, Ritz J, Goldstein G, Schlossman JF: Ina determinants on human T cell subsets defined by


34. Velardi A, Cooper MD: An immunofluorescence analysis of the ontogeny of myeloid, T and B lineage cells in mouse hemopoietic tissues. J Immunol 133:673, 1984
Acute leukemias associated with the 4;11 chromosome translocation have rearranged immunoglobulin heavy chain genes

WM Crist, ML Cleary, CE Grossi, EF Prasthofer, GD Heggie, GA Omura, AJ Carroll, MP Link and J Sklar