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

Studies of acute leukemia with the 4;11 translocation have yielded conflicting results regarding the lineage of the cell of origin in this disease. To investigate this issue further, we have examined the state of immunoglobulin genes in tumor cells from two affected patients, immunophenotyped their leukemic cells using a number of monoclonal antibody reagents with specificities for lymphoid or myelomonocytic antigens, and examined the malignant cells by electron microscopy. DNA was extracted from leukemic bone marrow cells and hybridized with radiolabeled DNA fragment probes specific for the constant region of immunoglobulin heavy chain and \( \alpha \) and \( \lambda \) light chain genes. Autoradiographs revealed rearrangement of both allelic heavy chain genes, but a germ-line configuration of light chain genes in both cases. Surface marker analysis showed that blasts from both patients expressed HLA-DR and the myeloid antigens Leu-M1, 1C2, 2D1, and 4B3, but lacked 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 blast cells 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.

A C U T E L E U K E M I A associated with the 4;11 chromosomal translocation occurs most frequently in infants and young adults and has a poor prognosis.1-3 Hyperleukocytosis and splenomegaly are frequently present. Morphological, cytochemical, ultrastructural, and immunologic features consistent with early myelomonocytic or lymphoid lineage have been described for blasts from individual patients and for blasts from the same patient.7 The blast cells from affected patients do not express surface or cytoplasmic immunoglobulin (\( \text{slg} \) or \( \text{clg} \)), nor do they form E rosettes with sheep erythrocytes (ESR). Most do not bear the common acute lymphocytic leukemia antigen (CALLA). They often express HLA-DR and P24/BA-2 antigens and occasionally express the BA-1 antigen. Terminal deoxynucleotidyl transferase (TdT) has been detected in some cases.7

Most patients appear to have lymphoid leukemia by standard morphologic criteria, although cells from a few display monocytoid features. Cytochemical stains have occasionally revealed periodic acid-Schiff (PAS) block positivity, and the blasts fail to stain with myeloperoxidase.5,7 Ultrastructural features consistent with “dysplastic” myeloid cells have been noted in blasts from some affected subjects.7 However, the nature of these leukemias remains controversial since none of the features noted has been definitive for lineage assignment of the malignant cells. Taken altogether, the evidence available suggests that the cell of origin is a very undifferentiated WBC progenitor.

In germine cells, the variable and constant domains of each type of immunoglobulin polypeptide chain are encoded as separate gene segments in DNA distantly linked within the respective chromosomal loci. Prior to immunoglobulin synthesis, these gene segments must undergo a series of somatic recombination events within individual B cells. These events bring a variable region gene segment into close proximity with a constant region segment, resulting in the formation of a transcriptionally active immunoglobulin gene. The altered configuration of immunoglobulin gene segments with respect to that of germline cells provides a molecular marker for B cell development, as rearrangement of immunoglobulin gene segments appears to be largely restricted to cells of B lineage.5,9

In order to investigate further the lineage of the acute leukemia associated with a 4;11 chromosome translocation, we have studied two affected patients, using immunologic, ultrastructural, and cytochemical techniques, and have also investigated the state of the immunoglobulin genes within their blast cells. We found that the blasts from both patients had rearrangement of both allelic heavy chain genes and expressed HLA-DR antigen but not CALLA. This phenotype would be consistent with an early B progenitor cell. However, in both patients, malignant cells also expressed certain myelomonocytic antigens.

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 \( \alpha \)-naphthyl esterase)
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 chloracetate esterase and α-naphthyl acetate esterase by standard techniques. TdT antiserum followed by a goat anti-rabbit IgG-FITC conjugate (Pharmacia P-L Biochemicals, Milwaukee). Cytocentrifuge cell preparations were fixed for 20 minutes in cold methanol prior to immunofluorescent staining.

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 Cγ and mixed Cγ light chain
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 ($\times 10^9$/L)</th>
<th>Survival (mo)</th>
<th>Time of Study</th>
<th>No. of Cells Examined</th>
<th>Banded Karyotypes</th>
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<tr>
<td>1</td>
<td>22 yr/M</td>
<td>0</td>
<td>30.0</td>
<td>5.5</td>
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<td>16</td>
<td>4, 12</td>
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<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>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Relapse</td>
<td>21</td>
<td>19</td>
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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...
nonleukemic marrow elements present in the specimen. In contrast, all light chain genes were present in their germline configurations (Fig 2).

**DISCUSSION**

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 1 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 progenitor. 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 al 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 la-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 Ia/B4-positive subgroup (stage II). None of the stage I patients expressed C. Results of analysis 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 myelocytic 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. 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. 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 characterized in T cell neoplasia, no example of V region recombination 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 additional corroborating evidence is necessary.

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

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**Table 2. Laboratory Findings**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>FAB Type</th>
<th>TdT</th>
<th>HLA-DR</th>
<th>CALLA</th>
<th>B4</th>
<th>Leu-9</th>
<th>Leu-5</th>
<th>clg</th>
<th>sig</th>
<th>E Rosette</th>
<th>Myeloid Markers*</th>
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<tbody>
<tr>
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<td>L1</td>
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<td>&lt;1</td>
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</tr>
<tr>
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<td>90</td>
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<td></td>
<td></td>
<td></td>
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<td>80/0.5</td>
<td>79</td>
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<table>
<thead>
<tr>
<th>Lymphoid Markers*</th>
<th>Myeloid Markers*</th>
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</thead>
<tbody>
<tr>
<td>E Rosette</td>
<td>Leu-M1</td>
</tr>
<tr>
<td>80</td>
<td>36</td>
</tr>
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</table>

FAB, French-American-British classification; L1, common morphology of childhood ALL; MMA, myelomonocytic antigen; CALLA, common acute lymphocytic leukemia antigen; clg, cytoplasmic immunoglobulin; sig, surface immunoglobulin; TdT, terminal deoxynucleotidyl transferase.

*Percentage of reactive cells by IFA determination.

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**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 (Ck) gene analysis, the DNA was cleaved with the restriction endonuclease EcoRI. For light chain gene analysis (Ck) the DNA was cleaved with the BamHI restriction enzyme. Under our hybridization conditions, a faint cross-hybridizing band of unknown significance is detected with the JH probe as noted in lane 1 and as we have previously published. Arrows denote the positions of rearranged immunoglobulin bands: lines indicate the position of germline 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-\(^2\), sIg-, CALLA-, Ba-1-, Ba-2-, but HLA-DR\(^+\) and TdT\(^+\). Six of these patients who were studied for sIg 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.\(^2\) 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.\(^1,3\) 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.\(^4\) 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.\(^4\) Alternatively, it is possible that the cell of origin of these leukemias is a common progenitor for B cells and myelomonocytic cells.

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Acute leukemias associated with the 4;11 chromosome translocation have rearranged immunoglobulin heavy chain genes

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