This report describes the clinical and laboratory features of seven cases of acute leukemia associated with the 4;11 chromosomal translocation. All seven children had acute lymphoblastic leukemia by standard morphologic and cytochemical criteria. Leukemic blasts from six of seven patients were terminal deoxynucleotidyl transferase–positive. Immunologic phenotyping suggested the leukemias were of B cell origin; blasts from five patients expressed HLA-DR and p24 (CD-9 antibody), blasts from three patients expressed B4 (CD-19), and blasts from two patients expressed the common acute lymphoblastic leukemia antigen (CD-10). One patient’s leukemic blasts contained cytoplasmic immunoglobulin. Analysis of DNA from four of five patients demonstrated additional evidence of B cell differentiation with heavy-chain immunoglobulin gene rearrangement. When DNA from the four patients with heavy-chain immunoglobulin gene rearrangement was analyzed, one patient’s DNA demonstrated light-chain immunoglobulin gene rearrangement. However, flow cytometric analysis of blasts from three patients showed the simultaneous expression of the lymphoid-associated antigen B4 (CD-19) and the myeloid-associated antigen My-1 (X-Hapten). Electron microscopic examination of blasts from one patient that expressed both lymphoid- and myeloid-associated antigens demonstrated ultrastructural characteristics of both lineages. These findings suggest that acute leukemia with the t(4;11) abnormality has mixed lineage characteristics as a result of leukemogenesis in a multipotential progenitor cell or aberrant gene expression later in differentiation. Furthermore, serial analysis of karyotype, immunophenotype, and heavy-chain immunoglobulin genes revealed changes in these biologic markers over time, suggesting continued chromosome rearrangement and gene modulation after the leukemogenic event in cells with the t(4;11).

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The cells were postfixed in 1% osmic acid, dehydrated in increasing concentrations of ethanol, and embedded in Spurr. Unstained grids and grids stained with uranyl acetate and lead citrate were examined.

Chromosome analysis. Bone marrow cells were obtained from heparinized posterior iliac crest aspirates and were processed within 30 minutes of aspiration. Direct preparations were done in all instances, and in some cases they were supplemented by 24-hour unstained cultures. Early studies were performed with a modification of the technique of Tijo and Whang. More recently cases were studied with a direct bone marrow technique that includes treatment with trypsin and staining with Wright's stain to obtain G-banding.

Immunophenotyping and terminal transferase assay. Leukemic blasts were studied for rosette formation with sheep erythrocytes at 37 °C, and the results were considered positive if rosette formation was noted for >3% of blasts (confirmed on cytocentrifuge preparations). Bone marrow samples containing >80% leukemic cells were analyzed with a standard indirect immunofluorescence assay using monoclonal antibodies (MoAb) to identify cell surface antigens. Cells were first incubated in heat-inactivated, pooled human AB serum to eliminate Fc binding. Cells incubated with isotypic antibodies (MoAb) were used as negative controls. Lymphoid-associated antigens were identified with multiple antibodies representative of the cluster groups (CD) identified at the First or Second International Workshops on Leukocyte Differentiation Antigens and included p24 (CD-9), common acute lymphoblastic leukemia antigen (CALLA) (CD-10). B4 (CD-19), T-11 (CD-2), and T101 (CD-5). The HLA-DR antigen was detected by MoAb L243. When adequate cells were available, the presence of myeloid-associated surface antigens was assessed by a panel of markers including My-1, Mol (CD-11), SJ-D1 (provisional cluster, CDw14), SE1 (CDw14), and T5A7 (CDw17). Immunophenotyping also included an analysis for surface (slg) and cytoplasmic (clg) immunoglobulins. Terminal deoxynucleotidyl transferase (TdT) was identified by use of an indirect immunofluorescence assay.

Dual beam cytometric flow analysis. Two-color immunofluorescence measurements were carried out on a modified System 50H Cytophoerogr (Ortho Diagnostics, Westwood, Mass) equipped with an argon ion laser (model 164-05, Spectra Physics, Mountain View, Calif) and a tunable dye laser (model 590, Coherent, Palo Alto, Calif). The dye laser was pumped by an argon ion laser (Innova 90/5, Coherent) operating at 514 nm with an output power of 1 W. The argon ion laser operating at 488 nm (400 mW) excited fluorescein isothiocyanate conjugated (FITC) probes, whereas the dye laser was tuned to 590 nm (280 mW) for the excitation of Texas Red (TR) conjugated probes. The green (FITC) detection channel contained two bandpass (BP) filters in series: a 515-530 BP (Ortho) and a 515-525 BP (Oriel Corp, Stamford, Conn). A 625-635 BP filter (Becton Dickinson, Sunnyvale, Calif) was placed in the red detection channel. This combination of filters, in addition to the spatial separation of the images from the two beams, resulted in the complete absence of light from one fluorochrome at the detector for the other. Data was recorded and analyzed on a model 2150 computer (Ortho).

Indirect immunofluorescence was used for the determination of the presence of cell surface antigen by MoAb binding. All MoAbs were purified either by affinity or protein A- sepharose chromatography. The saturating concentration of MoAb was selected as described previously. FITC goat antimouse μ- or γ-chain-specific antibodies (Cappell Laboratories, Cochranville, Pa) were used as the second-stage reagent for the green detection channel. Biotinylated MoAbs were used for analysis using the red channel, with the second-stage reagent being TR-conjugated avidin (Tago Inc, Burlingame, Calif). The application of the biotinylated antibody always followed incubation with the FITC goat antimouse step.

Two-color immunofluorescence experiments were repeated, with each primary MoAb being detected by the alternate second-stage fluorochrome to assess the consistency of the measurements. These experiments, of necessity, required that the primary MoAbs be applied in reverse order. Isotype- or subtype-identical mouse myeloma proteins were used as controls at concentrations identical to the primary MoAb as determined by an enzyme-linked immunoabsorbent assay. Two-dimensional arrays were then constructed using an algorithm that computed the expected number of cells in each element resulting from nonspecific binding. The array corresponding to the appropriate control antibodies was then subtracted from the array for the test antibody.

Immunoglobulin gene analysis. For each patient, 20 μg of leukemic cell DNA was digested with the appropriate enzyme BamHI, HindIII, or EcoRI (3 U/μg DNA; Amersham Corp, Arlington Heights, Ill). The digested DNA was fractionated by electrophoresis on a 0.8% agarose gel, transferred to nitrocellulose paper according to the technique of Southern, and hybridized with nick-translated probes with specific activities of 2 to 5 × 108 cpm/μg DNA. The heavy-chain immunoglobulin gene probes included a 1.2 kb EcoRI fragment representing portions of the Cα1 and Cα3 domains and all of the Cμ2 domain or a 3.4 kb HindIII/EcoRI fragment of the Jμ region. A 2.5 kb germ line EcoRI fragment was used to analyze the k light-chain gene, and an 8.0 kb EcoRI fragment

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*Edge palpable below costal margin.
and on all seven children. The median age was 3.7 years (range, male. Three children (nos. 1, 6, and 7) had massive hepato-

leukemia that the incidence of this abnormality in childhood acute ing leukemia complete conditions were used for hybridization and washing, the filters were

After high-stringency used to analyze the X light-chain gene.39

t(4;11)(q21;q23)

6 Diagnosis 46,XX,t(4;11)(q21;q23)

4 Diagnosis 46,XY,t(4;11)(q21;q23)

3 Diagnosis 48,XX,+21,+22,t(4;11)(q21;q23)

2 Diagnosis 45,XX,−20,t(2;16)(p13;p13)

1 Diagnosis 46,XX,t(4;11)(q21;q23)

2nd Relapse 46,XX,t(4;11)(q21;q23),i(7q)

3 Diagnosis 48,XX,+21,+22,t(4;11)(q21;q23)

4 Diagnosis 46,XY,t(4;11)(q21;q23)

Relapse 47,XY,+X,inv(2p)72q13, −4, + der(4)t(4;7)(q35;?), t(4;11)(q21;q23),t(6;9)(q25;q22),t(12;19)(q23;p13)

5 Diagnosis 46,XY,t(4;11)(q21;q23),inv(16)(p13q22)

6 Diagnosis 46,XX,t(4;11)(q21;q23)

7 Diagnosis 46,XX,t(4;11)(q21;q23)

Relapse 46,XX,t(4;11)(q21;q23)

used to analyze the λ light-chain gene.38 After high-stringency conditions were used for hybridization and washing, the filters were washed, dried, and exposed at −70 °C to XAR-5 films (Kodak, Rochester, NY) for one to five days in the presence of intensifying screens. All experiments included control DNA from normal mono-nuclear cells, tonsil or spleen.

RESULTS

Incidence. Of the 340 children with acute leukemia for whom chromosome analyses were performed, 237 had complete banding. The 4;11 translocation was identified in leukemic cells from six of these patients at diagnosis, indicating that the incidence of this abnormality in childhood acute leukemia is at least 2.5%.

Laboratory findings. Table 1 presents the clinical data on all seven children. The median age was 3.7 years (range, 0.3 to 14 years). Five children were female, and two were male. Three children (nos. 1, 6, and 7) had massive hepatosplenomegaly at diagnosis. The four patients (nos. 1, 4, 6, and 7) with extreme leukocytosis (median, 310 × 10^9/L; range, 210 to 346 × 10^9/L) had only the t(4;11) karyotypic abnormality detected at diagnosis (Table 2). The three patients (nos. 2, 3, and 5) with distinctly lower leukocyte counts (median, 27.3 × 10^9/L; range, 12.1 to 27.8 × 10^9/L) had associated karyotypic abnormalities and may represent a clinically distinct subgroup of patients with t(4;11). The hemoglobin concentrations (range, 2.3 to 11.7 g/dL) and platelet counts (range, 10 to 159 × 10^9/L) were variable in these patients. Three patients (nos. 1, 2, and 7) had overt CNS disease with leukemic cells in their spinal fluid. No patient had a mediastinal mass, and only one had significant adenopathy. By light microscopic examination, the leukemic blasts from all seven patients were morphologically lymphoid (FAB L1 or L2) without MPO, SBB, CAE, or ANB staining.

Six of the seven children demonstrated the t(4;11) (q21;q23) chromosomal rearrangement at the time of diagnosis, whereas patient 2 exhibited this abnormality only at relapse (Table 2). In patient 1 the t(4;11) was not detected at relapse, but patients 4 and 7 demonstrated this abnormality at both diagnosis and relapse. Three out of 4 patients had different karyotypes at relapse, and patient 2 had karyotypic changes between diagnosis, the first, and the second hematologic relapse. In four of the seven patients, the t(4;11) was associated with other chromosomal abnormalities either at diagnosis (patients 3 and 5) or at relapse (patients 2 and 4). No patient demonstrated an extra chromosome number 14 at diagnosis or at relapse.

The results of immunophenotyping and TdT analysis are presented in Table 3. A large percentage (60% to 98%) of leukemic blasts from five of the six patients tested expressed TdT activity at the time of diagnosis. Blasts from all six children that could be studied at diagnosis expressed

<table>
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</tr>
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<td>Relapse</td>
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<td>5 Diagnosis</td>
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<td>6 Diagnosis</td>
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<td>S1F1 (CDw14)</td>
</tr>
<tr>
<td>T5A7 (CDw17)</td>
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Abbreviations: Dx, diagnosis; ND, not determined.
*Positive when tested by flow cytometry (see text).
All results given as percent positive cells determined by fluorescence microscopy.
HLA-DR, whereas four expressed p24 (CD-9). No blasts formed E rosettes or reacted with anti-T cell MoAbs. Blasts from patient 4 contained cIg at diagnosis and relapse, whereas the blasts from patient 2 expressed CALLA (CD-10) at diagnosis but not at the first or second hematological relapse. All three patients tested (nos. 4, 6, and 7) expressed the B lineage–associated surface antigen B4 (CD-19).

Cells taken at diagnosis from four patients were examined for the presence of myeloid-associated antigens. Two of these four (nos. 4 and 7) showed definite positive subpopulations (>15%). Patients 4 and 7 with My-1+ blasts have relapsed and their blasts again expressed this antigen. Blasts from patient 2 were not tested at diagnosis but were My-1+ at the first and second relapse. There were adequate cells remaining from the diagnostic specimens of three patients (nos. 4, 6, and 7) to allow detailed studies using flow cytometry. Using single-fluorochrome analysis, histograms demonstrated definite My-1 (X-Hapten) and T5A7 (CDw17)-positive subpopulations when compared with cells incubated with the control myeloma protein, and the ratio of My-1+ cells to My-1+ cells was always greater than unity (range, 1.1 to 2.3). The majority of the blasts from these three patients were also positive for the pan–B cell antigen B4 (≥85%). Dual beam immunofluorescence was then used to demonstrate that
virtually all My-1+ cells expressed B4 and greater than 90% of the My-1+ cells were also T5A7+ (Fig 1). The B4-positive cells also simultaneously expressed the p24 (CD-9) and HLA-DR antigens (data not shown). In the two-color experiments, the use of alternate fluorochromes, as explained in Materials and Methods, did not affect the results.

Ultrastructural studies on patient 7 were consistent with the dual-beam immunofluorescence studies. The predominant blasts appeared lymphoid with a round-to-oid nucleus, peripherally margined chromatid, and one or two prominent nucleoli. The cytoplasm contained variable numbers of mitochondria, occasional short strands of rough endoplasmic reticulum, and polyribosomes. Approximately 25% of these lymphoid-appearing blasts contained electron-dense, membrane-bound granules that were peroxidase-positive (Fig 2). In addition, there was a population of blasts that was monocytoid, comprising approximately 15% to 20% of the blast cell population. These cells were larger than the lymphoid-appearing blasts and contained nuclei that were infolded with evenly distributed chromatin and a prominent nucleolus. In these cells the cytoplasm was more abundant and contained an occasional Golgi apparatus as well as numerous small electron-dense granules, some of which were peroxidase positive. Extensive examination of multiple grids failed to reveal a population of cells that exhibited dysplastic myeloid features or contained basophil/mast cell granules.

Four of five patients tested at diagnosis (nos. 1, 2, 4, and 6) showed evidence of heavy-chain immunoglobulin gene rearrangement (Table 4). Patient 6 had only a single rearranged μ gene, whereas three of these patients (nos. 1, 2, and 4) demonstrated more than two bands that hybridized to the μ constant-region probe. When the results were analyzed using a densitometer, the DNA from patient 1 had three bands (one germ line, two rearranged), and the DNA from patient 4 had at least four bands (one germ line, three rearranged) that hybridized to the Cμ probe after BamHI digestion (Fig 3). Patient 2 demonstrated distinct differences in karyotype and immunophenotype between diagnosis and each relapse (Tables 2 and 3). This patient also demonstrated distinct changes in the sizes of the Μ-chain gene rearrangements. When analyzed by BamHI digestion, four μ constant-region–hybridizing bands were present at diagnosis, but at first hematologic relapse only three were present (Fig 3). Analysis of the DNA from patient 2 at the time of first relapse by HindIII digestion and Jμ probing revealed a far more complex pattern than that seen by BamHI digestion, with ten bands being apparent on the autoradiogram (Fig 4). Of these, 9 are rearranged and 1 is germ line. This pattern is reproducible and probably does not represent underdigestion of DNA. When DNA from the second relapse was examined by HindIII digestion and Jμ probing, a much simpler pattern appeared, with three rearranged and one germ line μ genes being observed (Fig 4). It is clear that two of the rearranged bands represent the majority of the Jμ-hybridizing material in this sample. The simultaneous analysis of the first and second relapse on the same gel clearly demonstrates conservation of some rearranged heavy-chain bands but the elimination of others. Analysis of DNA from patient 4 at the time of diagnosis using HindIII digestion and Jμ probing also revealed a far more complex pattern than that seen using
Fig 3. (A) Schematic representation of the human Ig gene region with restriction endonuclease sites: E, EcoRI; H, HindIII; and B, BamHI. The Cμ probe consisted of a 1.2-kb EcoRI fragment subcloned into pACYC184 and contains the complete Cμ 2 domain and portions of Cμ 1 and Cμ 3. The Jμ probe consisted of a 3.3-kb BamHI/EcoRI fragment subcloned into pBR322. (B) Southern blot analysis of the Ig gene. DNA was digested with BamHI, and blots were hybridized with a Cμ-specific probe. The results for cases 1, 2, (D, diagnosis; R, 1st relapse), 4, 6, and 7 are shown (lanes 1 to 6, respectively). Lines represent the positions of the 17-kb germ line gene in the gels. Arrows indicate the positions of rearranged bands (determined by densitometer).

Southern blots were washed to remove bound µ probe and rehybridized to a κ light-chain constant-region probe. None of the four patients with heavy-chain rearrangements had a rearranged κ light-chain gene (Table 4). The arrangement of the λ light-chain genes was examined using EcoRI digests, and one patient (no. 6) was found to have a λ light-chain gene allele rearranged. This was of interest since the κ-chain genes were apparently in the germ line configuration and κ-chain genes rearrange before λ in the Ig gene hierarchy. Close examination of the κ-gene blot for this patient revealed that the intensity of hybridization was lower than the control DNA, indicating that perhaps one κ allele was deleted, although the second was in the germ line configuration. We conclude that, as found in several patients by Korsmeyer et al, one κ-gene allele has been deleted and the rearrangement of the λ allele therefore does not violate the hierarchy of gene rearrangements.

Treatment outcome. All patients achieved complete remissions that ranged from 4+ to 30 months in duration. Patients 3, 5, and 6 remain in their first clinical remission 22+, 4+, and 4+ months, respectively. Patient 2 had a first remission that lasted 24 months and a second remission that lasted 12 months. The three patients in this report who died from progressive leukemia had survivals of 12, 14, and 41 months. Three of the four patients in whom a hematologic relapse developed achieved a second complete remission without difficulty.

DISCUSSION

We studied seven patients whose leukemic blasts had translocations between the long arms of chromosomes 4 and 11. Their ages ranged widely, and in contrast to several earlier reports, their leukemic blast cells were clearly FAB-type L1 or L2. Only four patients had extreme hyperleukocytosis, a common finding in most series. Anemia and hepatosplenomegaly were not consistent features in our patients, and none presented with a mediastinal mass.

The majority of t(4;11)-positive patients reported in the literature achieved complete remissions; however, their remission lengths have seldom exceeded 8 months. Whether the extended periods of complete remission in three
of our patients reflect differences in other biologic characteristics or the use of more intensive therapy cannot be answered with certainty because of the small number of patients with the t(4;11).

The presence of associated karyotypic abnormalities in our patients is unusual and may be associated with important clinical characteristics such as a lower-median leukocyte count. The associated cytogenetic abnormality in patient 5 is particularly interesting since the inversion of chromosome 16 (p13q22) has been associated with nonlymphocytic leukemia. The blast cells of three of four patients analyzed both at diagnosis and relapse showed karyotypic evolution, consistent with a previous report suggesting that karyotypic evolution may be common in patients with the t(4;11). Not unexpectedly, we were able to show gene rearrangement and immunophenotypic evolution coincident with the observed chromosomal changes.

For the three cases in which adequate cells were available to allow extensive studies of surface antigen expression, greater than 85% of the blasts were positive for the pan-B cell antigen B4 (CD-19). This antigen is expressed early in B cell differentiation and is restricted to cells of the B lineage. Subpopulations of B4* cells that expressed the p24 epitope (CD-9) also expressed the antigens identified by the MoAbs My-1 and TS4A7. The molecule bearing the p24 epitope has been found on the surface of non-T, non-B ALL blasts and platelets as well as cells of monocytoid and lymphoid lineages. MoAb My-1 detects a determinant (X-Hapten) expressed on the majority of myeloid cells and a subset of monocytes. The reactivity of this MoAb appears to be restricted to nonlymphoid cells. The TS4A7 MoAb is reactive with a lactosylceramide moiety that is the common precursor for glycosphingolipids of the lactoseries, ganglioseries, and globoseries. This antibody has been shown to bind to cells of the myeloid lineage, monocytes, phytohemagglutinin-stimulated lymphocytes, and approximately one third of non-T, non-B ALL blasts. In three patients, we demonstrated simultaneous expression of a B lymphoid antigen (B4) and several lymphoid-associated antigens with a nonlymphoid antigen (My-1). These patients appear to have acute mixed-lineage leukemia (AML/LL), a finding consistent with reports of the t(4;11) in leukemic cells with lymphoid and myeloid characteristics. A recent review of cases of hybrid acute leukemia suggested that simultaneous expression of myeloid and lymphoid characteristics may be associated with the t(4;11). Of the 18 cases included in this review as hybrid leukemia, seven contained the t(4;11). Additionally, a recently described cell line containing the t(4;11) has both myeloid and lymphoid features, similar to the cases we describe here.

The ultrastructural and ultracltochemical analysis of blast cells from patient 7 demonstrated both lymphoid and nonlymphoid characteristics. Thus, in this patient there appears to be a morphologic correlation with the immunologic identification of both lymphoid and nonlymphoid antigens on the same blasts. The ultrastructural features of this patient are similar to some of the previously described patients with acute leukemia characterized by the t(4;11) translocation. In the series reported by Parkin et al, blasts from eight of the ten patients exhibited predominantly lymphoid morphology. However, in one of the three patients tested, the lymphoid-appearing blasts contained peroxidase-positive granules.

Blasts from three of the five patients analyzed for immunoglobulin gene configuration demonstrated only heavy-chain rearrangements, whereas a fourth demonstrated both heavy- and light-chain gene rearrangements, suggesting a normal progression of B cell differentiation. Although the finding of rearranged heavy-chain genes is not specific for leukemia of lymphoid lineage, it is strongly B cell associated and provides indirect evidence for a lymphoid differentiation of the leukemic blasts.

It is unusual and potentially important that three of the five patients we tested (nos. 1, 2, and 4) had greater than two DNA bands hybridizing with the immunoglobulin heavy-chain gene probe (Table 4, Figs 3 and 4). The presence of more than two rearranged \( \mu \)-chain genes has been observed in transformed lymphoid cells and in other leukemia patients. More than two hybridizing bands could have resulted from additional alleles of the \( \mu \) gene on extra copies of chromosome 14; however, we could not identify an extra chromosome 14 in any of our patients. Alternatively, multiple bands could result from more than one transformed clone, and therefore, such cases may represent biclonal leukemia. A third explanation is that multiple bands are a result of evolution within the leukemic cell population. This hypothesis is supported by the finding of karyotypic evolution at relapse in the three patients having more than two heavy-chain immunoglobulin gene bands (Table 2). Further support for evolution in the leukemic population comes from our findings of coincident changes in karyotype, immunophenotype, and the heavy-chain immunoglobulin gene rearrangement at diagnosis and at relapse in one patient (no. 2). The pattern of rearrangements in patient 2 at first relapse (nine rearranged, one germ line) suggests the presence of five distinct sublines (Fig 4). This is reminiscent of the continued rearrangement of Ig heavy-chain genes that occurs in Abelson murine leukemia virus-transformed cells in which up to six sublines have been observed to arise from a clonal population over several months in culture. A relationship between the evolution of biologic characteristics and relapse is purely speculative, but provocative. If such a relationship exists, it will be clarified by larger studies.

In each of our cases, the break point on chromosome 11 was within or near bands q23–q24. Recent localization of the \( c \)-ets oncogene on chromosome 11 at this position suggests that \( c \)-ets could be involved in the pathogenesis of some cases of acute leukemia. It will be interesting to examine t(4;11)-positive blasts for activation of the \( c \)-ets oncogene, which may be important for leukemogenesis.

ACKNOWLEDGMENT

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Clinical and laboratory characteristics of acute leukemia with the 4;11 translocation

J Mirro, G Kitchingman, D Williams, GJ Lauzon, CC Lin, T Callihan and TF Zipf