Cellular and Molecular Studies on Infant Null Acute Lymphoblastic Leukemia

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We have studied the cellular and molecular basis of eight cases of infant null acute lymphoblastic leukemia (ALL). All eight patients were under 9 months of age and presented with leucocyte counts in excess of $6 \times 10^9$/L, organomegaly, and in two cases CNS infiltration. Although seven cases were morphologically classified as ALL, one patient had both lymphoid and myeloid features. Phenotypic analysis of leukaemic blasts from all patients showed a typical null ALL pattern, i.e., CD10 (common ALL antigen)-negative, strongly HLA-DR-positive, and CD19 (B4)-positive. The presence of terminal deoxynucleotidyl transferase (TdT) at presentation was positive in six patients' cells and negative in two. Two patients also expressed the myeloid-associated markers CD33 (MY9) and CD15 (TGI), and coexpression of CD19 and CD33 was confirmed in these two by using dual marker flow cytometry (fluorescence-activated cell sorting). Electron microscopic examination of the same two patients' cells showed the presence of monocyoid blasts labeled with the pan-B cell antibody B4 (CD19). Short-term culture of one of these patients indicated this fact.

A high proportion of cases of leukemia in infants under 1 year of age are of the “null” acute lymphoblastic leukemia (ALL) phenotype, that is, the blast cells lack the common ALL antigen (CALLA) but usually express HLA-DR and are often terminal nucleotidyl transferase (TdT) positive.1,2 A number of patients have also been shown to express monoclonal antibody–defined surface markers associated with myeloid or monocyoid cells.3 Such patients characteristically present with a high leucocyte count, organomegaly, and early CNS disease and have a particularly poor prognosis.4 Cytogenetic analysis in patients where all these characteristics are present show involvement of band 11q23 in a high proportion of cases.5 The translocation t(4;11) is the most typical and occurs in about 56% of cases.6,7 Other karyotypic changes involving band 11q23-25 such as t(11;19) and t(9;11), both more commonly associated with myelomonocytic leukemia, have also recently been described in infant ALL.3,7 The expression of mixed phenotypic character together with the results of ultrastructural studies in patients with t(4;11) would suggest that the leukemic population derives from a stem or multipotent precursor cell capable of either lymphoid or myeloid differentiation.3,8 Immunoglobulin gene rearrangement studies have shown that blast cells from the majority of patients studied have rearranged heavy-chain genes, thus providing strong evidence for commitment to the B lineage.9,10 We have therefore studied the blast cells from eight unscreened infants by using a combination of cellular and molecular techniques. Our data indicate that infant null ALL probably derives from a precursor cell sharing the phenotypic and genotypic features of both B and myeloid progenitor cells.

Materials and Methods

Patients. Eight infants, all less than 9 months of age, who presented with acute leukemia, consecutively over a 27-month period at the Hospital for Sick Children were studied. Two patients were also studied at relapse. In all cases the blasts were morphologically classifiable as lymphoblastic leukemia (L1 or L2), but in one case monocyoid features were also present. Bone marrow smears were stained with May-Grünwald-Giemsa (MGG), PAS, Sudan black (SB), and nonspecific esterase (NSE). Morphological classification followed the French-American-British (FAB) criteria. Informed consent was obtained from the parents of all patients prior to commencement of these studies and with the approval of the Ethical Committee of the Hospital for Sick Children.

Immunophenotyping. Bone marrow samples were collected into Hanks' buffered saline solution containing preservative-free heparin. Mononuclear cells containing >90% blasts were separated on 60% Percoll (Pharmacia, Uppsala, Sweden) and analyzed by indirect

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immunofluorescence using a panel of monoclonal antibodies to human leukocyte differentiation antigens as defined by the Third International Workshop on Human Leucocyte Differentiation Antigens, Oxford, 1986. These included CD10 (CALLA, AL2), CD19 (B4), CD2 (OKT11), CD7 (WT1), CD13 (MY9), CD15 (TG1), CD13 (MY7), and anti-HLA-DR (Royal Free B [RFB] HLA-DR). Cytosin preparations of leukemic blasts fixed in methanol were also analyzed for cytoplasmic IgM by using a directly conjugated rabbit antihuman IgM (Kallestad Laboratories, Inc, Austin, TX) and TdT (SuperTechs, Bethesda, MD) as described by Bol- 

Two-color flow cytometry (fluorescence-activated cell sorting). Two-color FACS analysis was carried out on cryopreserved cells from patients 3 and 4. Antibodies of different immunoglobulin isotypes were used at predetermined saturating concentrations either as purified antibody or ascitic fluid. Cells were incubated in the order specified, with CD19 (B4, IgG1) followed by fluorescein isothiocyanate (FITC)-labeled goat antimouse IgG1 (Southern Bio- 
techology Associates, Birmingham, AL) and then CD33 (MY9, IgG2a) directly coupled to phycoerythrin (Coulter Immunology, Hialeah, FL) for 20 minutes each at 4°C. Between each labeling, the cells were washed with phosphate-buffered saline (PBS)/0.1% bovine serum albumin (BSA) and finally resuspended in the same medium for the analysis. The cells were analyzed on an Imperial Cancer Research Fund (ICRF) cell sorter that had been modified for analysis on the three parameters of forward light scatter, fluorescein fluorescence, and phycoerythrin fluorescence. The laser output was 200 mW and the excitation wavelength, 488 nm. Photomultiplier voltages and preamplifier gains were set at 800 to 850 V and 8 and 16 for red and green fluorescence detection. Emitted light to each photomultiplier tube was split and filtered with a 550-nm short-pass filter for FITC and a 575-nm long-pass filter for phycoerythrin. Electronic compensation to allow for red/green signal separation was done by labeling the cells separately with either the FITC anti-IgG1 or phycoerythrin alone.

Ultrastructural studies. Electron microscopic analysis was carried out on cryopreserved leukemic blast cells from patients 3 and 4. Cell pellets were fixed with 2.5% glutaraldehyde in 0.067 mol/L cacodylate buffer, pH 7.3, for two hours. After a brief rinse with fresh cacodylate buffer the pellets were postfixed in 1% osmium tetroxide in cacodylate buffer for a further hour. Dehydration in fresh cacodylate buffer was carried out over a period of four hours including two hours in two changes of absolute ethanol. After an overnight soak in a 50:50 epoxidepyrone and araldite resin mixture, the pellets were embedded in fresh araldite epoxy resin at 60°C for 48 hours. Sections were cut on a Reichert OMEU4 (Reichert Jung, Cambridge, UK) and examined in a Zeiss EM10 electron microscope. Grid staining was performed, where necessary, with lead citrate and uranyl acetate.

Immunoelectron microscopy. The cells were first incubated in a nonspecific blocking medium of 4% normal goat serum and 1% BSA in PBS, pH 7.2, for one hour at 4°C. The same medium without goat serum was then used to make appropriate dilutions of the primary antibody B4, and samples of cells from both patients were then incubated in antibody for two hours at room temperature. After three washes with PBS, the cell samples were incubated for a further hour at room temperature in the second antibody, a 1:50 dilution of goat antimouse serum conjugated to 15-nm colloidal gold particles (Janssen Pharmaceuticals, Beere, Belgium) in the same diluent as that used for the primary antibody. The cells were then washed three times before processing for electron microscopy as described in the previous paragraph.

Clinicopathological findings. Cryopreserved cells from patient 3 were established in short-term culture in RPMI medium (Flow Laborato- 
ries, Ryde, UK) plus 10% fetal calf serum (FCS). Cells were seeded into 60-mm Petri dishes at a concentration of 5 x 10^4/mL and treated with 2.5 ng/mL 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma Chemical Co, Poole, UK). Control cells received no TPA. After 48 hours at 37°C, TPA and control cells were harvested and repopulated and cytospin preparations were stained with MGG, PAS, SB, and NSE. Phagocytosis was performed by incubating both TPA-treated and control cells as cell pellets with ten washed yeast particles per cell. The cells plus yeast particles were incubated in the presence of 10% (vol/vol) fresh normal serum at 37°C for 20 minutes and then spun gently. The supernatant was discarded and the pelleted cells resuspended gently, spread onto glass slides, stained with Leishman’s, and examined for ingested yeast particles.

Cytogenetic analysis. All specimens, both bone marrow and peripheral blood, were processed as follows. Three separate cultures were set up in TC 199 (Wellcome Reagents, Beckenham, UK) supplemented with 20% FCS and glutamine (2 x 10^{-5} mol/L). One culture was incubated at 37°C for one to four hours before processing. Two cultures were incubated for 24 hours at 37°C; one of them was synchronized by adding fluorodeoxyuridine (FUDR) (10 μg/mL) and uridine (4 μg/mL) for 17 hours, followed by the addition of thymidine (10 μg/mL) and further incubation for six to seven hours. Colcemid was added for the last 15 minutes of incubation for the synchronized culture and the last hour of incubation for the other cultures. Processing was done by using hypotonic KCl (0.075 mol/L) for 15 minutes and fixing with chilled methanol/acetacid (3:1). The cell suspension was dropped onto cold wet slides and dried at 70°C. The slides were G-banded by immersion in a trypsin solution and stained with Leishman’s stain. A minimum of ten banded cells from each patient was fully analyzed.

DNA analysis. High-molecular weight DNA was extracted from the leukemic cells of all eight patients as previously described. Approximately 10 μg DNA was digested with the restriction enzymes EcoRI, BamHI, BglII, or HindIII (Boehringer, Mann-heim, FRG) according to the manufacturer’s instructions. A sample of placental DNA was always included as a control. The DNA fragments thus obtained were electrophoresed on 0.8% agarose gels and then transferred to nitrocellulose filters as described by Southern.31 The filters were then hybridized with random-primer–labeled probes having specific activities of 2 to 4 x 10^{6} cpm/μg DNA. The heavy-chain Ig gene probes consisted of a 1 kilobase (kb) EcoRI fragment containing the constant-region μ (Ca) domains of the H chain gene (kindly provided by Dr T. Rabbits) and both a 5.8- and a 2.5-kb fragment of the H chain joining region (JH, kindly provided by Drs P. Leder and W. Smith). Details of these probes have been published elsewhere.1,14 The JH 5' to the J region probe was a BamHI/BglII fragment excised from the intact 5.8-kb JH probe. The x light-chain probe was a 2-kb EcoRI fragment (kindly provided by Dr W. Smith).

T cell receptor genes were examined by using a 2.5-kb SacI/ 

RESULTS

Clinical findings. Table 1 shows the clinical data on the eight patients studied. The median age was 3.9 months (range, 1 to 8 months). Four patients were female, and four were male. All had hepatosplenomegaly at diagnosis, and two had massively enlarged kidneys. None had a mediastinal
mass seen on chest x-ray. All had leukocytosis with a mean WBC count of 285 × 10^9/L (range, 66.5 to 715), and two patients had CNS disease with leukemic blasts present in the CSF. All were morphologically classifiable as having ALL (L1 or L2) except for patient 3 in whom a mixed population of small blasts and large monocytoid cells were present. Patients 3 and 8 also showed a variable percentage of NSE-positive cells in the marrow.

Six patients were treated with daunorubicin, vincristine, prednisolone, and L-asparaginase and achieved remission, but two who were admitted in a moribund state were not given cytotoxic therapy.

**Immunophenotyping.** Cell surface marker data on the eight patients at presentation and on patients 1 and 7 at relapse are summarized in Table 2. Leukemic blasts from all eight patients failed to express CD10 (CALLA), CD2 (OKT11), and CD7 (WT1), with the exception of patient 3 whose blasts showed 32% CD7-positive cells. All were cytoplasmic TdT-negative but were strongly HLA-DR– and CD19 (B4)-positive. The cells from all but two patients were TdT-positive at diagnosis, and the cells of patient 1 were positive at relapse. Blast cells from patients 3 and 4 also both expressed a variable percentage of the myeloid-associated markers CD33 (MY9) and CD15(TG1). Two-color FACS analysis of these two patients' cells by using CD19 and CD33 antibodies showed that in both cases between 80% and 90% of the blasts coexpressed both markers (Fig 1).

**Ultrastructural studies.** At the ultrastructural level, bone marrow blasts from patient 3 showed the presence of

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**Table 1. Clinical Details**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex/Age</th>
<th>WBC</th>
<th>CNS</th>
<th>FAB</th>
<th>NSE</th>
<th>SB</th>
<th>PAS</th>
<th>Survival</th>
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<tr>
<td>1 (Dx)</td>
<td>F/4</td>
<td>470.2</td>
<td>-</td>
<td>L1</td>
<td>occ+</td>
<td>-</td>
<td>-</td>
<td>83% 16 mo</td>
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<tr>
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<td>+</td>
<td>L1</td>
<td>-</td>
<td>-</td>
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<td>50%</td>
</tr>
<tr>
<td>2</td>
<td>M/2</td>
<td>66.5</td>
<td>-</td>
<td>L1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>56% 17 mo+</td>
</tr>
<tr>
<td>3</td>
<td>F/1.5</td>
<td>120</td>
<td>-</td>
<td>M5/L2</td>
<td>30%</td>
<td>-</td>
<td>-</td>
<td>17% 3 d</td>
</tr>
<tr>
<td>4</td>
<td>M/1.5</td>
<td>220.3</td>
<td>+</td>
<td>L1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>83% 50%</td>
</tr>
<tr>
<td>5</td>
<td>F/8</td>
<td>122.4</td>
<td>-</td>
<td>L1</td>
<td>-</td>
<td>-</td>
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<tr>
<td>6</td>
<td>F/6</td>
<td>715</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>F/2</td>
<td>7.2</td>
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<td>L2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>L1</td>
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<td>-</td>
<td>5%</td>
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**Abbreviations:** Dx, diagnosis; Rel, relapse; ND, not determined; occ, occasional.

**Table 2. Surface Phenotype**

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<th>CD15</th>
<th>Cytoplasmic μ</th>
<th>TdT</th>
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<td>1</td>
<td>-ve</td>
<td>&lt;1</td>
<td>-ve</td>
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<tr>
<td>1 (Rel)</td>
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<td>&gt;95</td>
<td>80</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
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<tr>
<td>2</td>
<td>-ve</td>
<td>&gt;90</td>
<td>72</td>
<td>3</td>
<td>&lt;1</td>
<td>-ve</td>
<td>-ve &gt;90</td>
<td></td>
</tr>
<tr>
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<td>-ve</td>
<td>88</td>
<td>70</td>
<td>32</td>
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<td>1</td>
<td>23</td>
<td>71</td>
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<tr>
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<td>-ve</td>
<td>71</td>
<td>90</td>
<td>2</td>
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<td>-ve</td>
<td>80</td>
<td>80</td>
<td>2</td>
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<td>6</td>
<td>-ve</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>-ve</td>
<td>95</td>
<td>37</td>
<td>2</td>
<td>1</td>
<td>-ve</td>
<td>-ve &gt;90</td>
<td></td>
</tr>
<tr>
<td>7 (Dx)</td>
<td>-ve</td>
<td>95</td>
<td>68</td>
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<td>1</td>
<td>-ve</td>
<td>3</td>
<td>-ve</td>
</tr>
<tr>
<td>7 (Rel)</td>
<td>-ve</td>
<td>92</td>
<td>93</td>
<td>2</td>
<td>1</td>
<td>5</td>
<td>-ve</td>
<td>1</td>
</tr>
<tr>
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<td>-ve</td>
<td>95</td>
<td>95</td>
<td>2</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>3-4 95</td>
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**Abbreviation:** -ve, negative.
Fig 2. Electron micrographs of cells from patients 3 and 4. (A) Cells from patient 3 show indented nuclei and granules characteristic of the monocytic and myeloid lineage (original magnification ×7,600). (B) Cells from patient 4 show a mixed population, some with large round nuclei and the appearance of lymphoblasts and some with indented nuclei and monocytoid morphology (original magnification ×7,800). (C and D) Cells from patient 4 show monocytoid blasts labeled by the immunogold technique with CD19 (B4) antibody (original magnifications ×36,000 [C] and 110,000 [D]).
large cells with either multilobed or eccentrically placed nuclei, abundant cytoplasm, and ruffled edges characteristic of both myeloid and monocytoid cells at various stages of maturation. Very few lymphoblastlike cells were seen (Fig 2A). In contrast, the blasts from patient 4 showed what appeared to be a mixed population consisting of lymphoblasts characterized by round cells with a smooth surface, sparse cytoplasm, and relatively condensed chromatin and also cells of monocytoid appearance (Fig 2B). These latter cells however, were shown by immunogold labeling to bind the CD19 (B4) antibody strongly (Fig 2C and D).

**Induction experiments.** After treatment with 2.5 µg TPA and after 24 to 48 hours, the cells from patient 4 formed tight adherent clumps with cytoplasmic processes and ruffled edges characteristic of macrophages on the bottom of the Petri dish. Fifty percent of these cells were CD15 (TG1)-positive compared with 35% in the control. Both control and TPA-treated cells were strongly HLA-DR-positive, but in the TPA-treated cells the CD19 positivity fell to 10% (compared with 72% in the control). Morphologically the TPA-treated cells had the appearance of macrophages and metamyelocytes, and >95% were strongly NSE positive.

**Phagocytosis.** Examination of TPA-treated cells showed that 68% to 70% of the cells had ingested at least six yeast particles, whereas in the control cells only 40% had ingested the same number.

**Cytogenetic analysis.** At presentation, karyotypic analysis was performed on all patients except patient 5 but was not successful on patient 1, who was rekaryotyped at relapse. In all cases a common breakpoint was seen at band 11q23. The detailed karyotypes from all patients are shown in Table 3, and partial karyotypes are illustrated in Fig 3.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dx/Rel</th>
<th>BM/PB</th>
<th>Karyotype</th>
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<tr>
<td>1</td>
<td>Rel</td>
<td>PB</td>
<td>46,XX</td>
</tr>
<tr>
<td>2</td>
<td>Dx</td>
<td>BM</td>
<td>46,XX,t(11;19)(q23;p13.3)</td>
</tr>
<tr>
<td>3</td>
<td>Dx</td>
<td>BM</td>
<td>46,XY,del(1)(q34.2)dup(11)(p11.2→q23::q13→qter)</td>
</tr>
<tr>
<td>4</td>
<td>Dx</td>
<td>BM</td>
<td>46,XY,t(9;11)(p22;q23)</td>
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<td>Dx</td>
<td>BM</td>
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<tr>
<td>7</td>
<td>Dx</td>
<td>PB</td>
<td>46,XX</td>
</tr>
<tr>
<td>8</td>
<td>Dx</td>
<td>BM</td>
<td>46,XX,t(4;11)(q21;q23)</td>
</tr>
</tbody>
</table>

**Abbreviations:** BM, bone marrow; PB, peripheral blood.
DNA analysis. Immunoglobulin heavy-chain gene rearrangement was shown in all patients by using either the Cμ constant-region probe in conjunction with BamHI digestion of genomic DNA or the Jμ probe in conjunction with EcoRI or HindIII digestion. Patients 1, 2, 3, 5, and 6 all showed two rearranged and no germline bands that hybridized to the Jμ probe, whereas patient 8 showed a strong germline band and a single rearranged band. Patients 4 and 7 showed at least three rearranged bands plus a germline band (Fig 4A). Interestingly, these latter bands were only poorly resolved when using BamHI digestion and probing with the Cμ probe (Fig 4B). Patient 1 at relapse showed only a single rearranged band and a weak germline band, whereas blast cells from patient 7 at relapse showed an identical pattern of rearrangement to that at presentation. When DNA from the blast cells of all patients that was digested with HindIII was probed with the 5’-to-J-region probe, rearrangements of this region were seen in patients 1, 4, and 7 (Fig 4C).

No rearrangement of either the cβ1 or cβ2 region of the T cell receptor β chain was seen in DNA samples from any patient after DNA digestion with both EcoRI and HindIII (data not shown). κ Light-chain rearrangement was also absent in both BglII and BamHI digests of blast cell DNA (Fig 4D). Hybridization with a human c-ets cDNA probe after digestion of DNA samples from all patients with EcoRI failed to demonstrate rearrangements of ets in any case (Fig 5).

DISCUSSION

The eight unselected cases of infant null ALL described here presented consecutively to a single unit and show the typical clinical and laboratory features of ALL in infancy, namely, hyperleukocytosis, organomegaly, and CNS involvement. Immunophenotyping in all cases confirmed the early B cell features characteristic of these leukemias, that is, CD10 (CALLA)-negative but HLA-DR- and CD19-positive, which we and others have described previously. These observations have now been extended to the molecular level, and in agreement with Mirro et al and Crist et al, we found rearrangement of the immunoglobulin heavy-chain genes in all eight cases. With the exception of patients 4 and 7, who had multiple rearranged bands, the patterns of rearrangement in the other six indicated that only two alleles were involved. Kitchingman et al have suggested that the presence of more than two hybridizing bands could arise if more than one malignant clone is present as in biphenotypic leukemia, and this could be the case in patient 4. Patient 7 showed other translocations in the majority of her cells at the locus of the Ig heavy chain combined with the t(4;11), which could explain the multiple rearranged bands seen as a consequence of three cytogenetically distinct clones (Table 3). When DNA from the blast cells was probed with a BamHI/BglII fragment of the Jμ probe covering the 5’ to J region of the heavy-chain gene, retention, and rearrangements of this region were seen in patients 1, 4, and 7 (at both presentation and relapse). This region of the Ig heavy-chain gene has recently been shown to be primarily involved in heavy-chain rearrangements in both human leukemic T cells and a percentage of B precursor (null) ALLs and is thought to represent a primary or very early event in the commitment of a stem cell to the lymphoid lineage. The fact that three of eight of our patients’ cells showed this early rearrangement provides additional evidence for their commitment to the primitive B lymphoid compartment.

We found no evidence for κ light-chain rearrangement in blast cells from any of our patients, and our results are consistent with the observations of others who also found the CD10-negative, HLA-DR- and CD19-positive group of B precursor ALL to have germline κ and λ light-chain genes.
Fig 4. (A and B) Southern blot analysis of immunoglobulin heavy-chain gene rearrangement in leukemic blasts from patients 1 through 8 and control placental DNA (c) after digestion with (a) EcoRI and probing with the JH probe or (b) digestion with BamHI and probing with the Cμ probe. The germline band is indicated by a dash, and some but not all rearranged bands are indicated by arrows. Figures on the left side of each blot give the size in kilobases of a marker phage λ HindIII digest. Details of both the Cμ and the JH probes are given in the text. G, germline; R, rearranged; MR, multiple rearrangements. (C) Southern blot analysis of DNA from patients 1, 4, and 7 (at presentation and relapse) and control placental DNA (c) after digestion with HindIII and probing with the complete J region probe or the 5′-to-J-region probe. The germline band is indicated by a dash and is 10 kb in size, and some but not all rearranged bands are indicated by arrows. (D) Southern blot analysis showing the lack of κ light-chain gene rearrangement in DNA from patients 1 through 8 and control placental DNA (c) after digestion with BglII and probing with a Cκ light-chain probe. Only the germline band at 12 kb is seen.
abnormalities more usually associated with myeloid or myelomonocytic leukemia. In none of the cases we studied were the leukemic cells reactive with the early progenitor cell antibodies defined by the CD34 group. Dinndorf and Reaman also recently reported that blast cells from 12 infants with ALL failed to show any reactivity with myeloid-, megakaryocytic-, or stem cell–associated antibodies. No cytogenetic or molecular data were, however, given on these patients.

Cytogenetic analysis was performed on all of our patients and has shown a wider spectrum of translocations than those usually described in infant ALL but had in common changes involving band 11q23. Such uniformity in the karyotypes of an unselected series of patients with null ALL has not been previously documented, although this may be accounted for by the difficulty of detecting some of the more subtle rearrangements such as the t(9;11) and the t(11;19). The most consistent cytogenetic abnormality previously found in infant ALL has been the translocation t(4;11),3,6,11 which was present in only three of eight of our patients. Although the blast cells in the majority of cases of t(4;11) have been described as type L1, L2, or even L3,26 a number of reports have emphasized the myeloid features of the blast cells.3,8,10,11,27 Based on our own observations, we would suggest that the biphenotypic potential is not only confined to cases with the t(4;11) in that two of our patients whose blast cells coexpressed CD19- and CD33-associated markers had the translocations t(9;11) and t(11;19). The remarkable common feature in all our patients was chromosomal rearrangement of band 11q23, which suggests a common underlying genetic mechanism, although three different chromosomes and a duplication were involved in these translocations.

The human c-ets 1 oncogene has been mapped to band 11q23.25,32 We found no rearrangements of the c-ets 1 gene in the blast cell DNA from any of our patients, and our observations are consistent with those of others.29 A single report claiming to show rearrangements of c-ets in two cases of monocytic leukemia and small cell lymphocytic lymphoma remains to be substantiated.29 Preliminary unpublished observations from our own laboratory indicate that the c-ets 1 gene is, however, actively transcribed in the blast cells from patients with the 11q23 abnormality.

In summary, the fact that infant ALL very probably originates in a multipotent precursor cell in a similar way to chronic myelogenous leukemia, that this disease is frequently associated with chromosomal translocations involving band 11q23,3,30,31 and that the majority of patients affected are less than 1 year of age lead to the possibility that such leukemias may arise as a consequence of events connected with pregnancy, possibly triggered in utero.31 The nature of the genes or sequences involved at band 11q23 is intriguing and, in particular, how the nature of these sequences affects the differentiation of the tumor cells and the course and prognosis of the disease.

A folate-sensitive heritable fragile site at band 11q23 has been suggested by Secker-Walker et al and Pui et al possibly to be involved in the genesis of the disease. In a study of three of the patients presented in this paper, however, van
den Bergh et al. were unable to show any significant expression of a fragile site at 11q23 in cells from either the parents or the children studied when in hematologic remission. We therefore conclude that our data and that of others indicate that leukemias with karyotypic involvement of band 11q23 are heterogeneous with respect to the major cell type involved (lymphoid, myeloid, monocytoid) and that the clonogenic cell may be a stem cell capable of either lymphoid or myeloid differentiation. Further studies with more detailed molecular analysis of the translocations and rearrangements involved in this group of leukemias may help to clarify these issues and provide some insight into the etiology of the disease.

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REFERENCES


Cellular and molecular studies on infant null acute lymphoblastic leukemia

F Katz, S Malcolm, B Gibbons, R Tilly, G Lam, ME Robertson, B Czepulkowski and J Chessells

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