Evidence for Clonal Development of Childhood Acute Lymphoblastic Leukemia

By Lois W. Dow, Paul Martin, John Moohr, Michael Greenberg, Lorna G. MacDougall, Vesna Najfeld, and Philip J. Fialkow

To determine whether acute lymphoblastic leukemia (ALL) is a clonal disease and to define the pattern of differentiation shown by the involved progenitor cells, we studied the glucose-6-phosphate dehydrogenase (G6PD) types in the cells of 19 girls heterozygous for this X chromosome-linked enzyme. Lymphoblast immunophenotypes were those of HLA-DR+, CALLA+, ALL (six patients); pre-B cell ALL (two patients); T cell ALL (four patients); and undefined ALL (three patients). Malignant blast cells at diagnosis from ten patients displayed a single G6PD type, indicative of clonal disease. In contrast, both A and B G6PD in ratios similar to those found in skin were observed in morphologically normal blood cells from the same patients. The leukemic cells of three patients were examined at both diagnosis and relapse: in each instance the same G6PD type was found, consistent with regrowth of the original leukemic clone at relapse. Results of studies of cells from nine additional patients tested only at relapse were similar. Our results indicate that childhood ALL is a clonally derived disease involving progenitor cells with differentiation expression detected only in the lymphoid lineage.

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of females, one of the two X chromosome-linked genes for glucose-6-phosphate dehydrogenase (G6PD) is inactivated early in embryogenesis. Consequently, females heterozygous for the usual gene \( Gd^b \) and a variant, such as \( Gd^a \), have two populations of cells, one in which B-type G6PD is synthesized and one in which A-type enzyme is produced. In G6PD heterozygotes, normal cell populations contain a mixture of both cell types and exhibit both B- and A-type enzymes. Neoplasias of unicellular origin, by contrast, show only a single G6PD. It has been shown with the G6PD system that human hematopoietic neoplasias develop clonally.1,2

Results of earlier studies with G6PD of malignant cells from three girls and one woman with acute lymphoblastic leukemia (ALL) in relapse4 suggested that in each case the recurrent disease was clonal. Similar investigations indicate that Philadelphia chromosome-positive chronic myelogenous leukemia (Ph+ CML) and at least some cases of acute nonlymphoblastic leukemia (ANLL) involve multipotent stem cells capable of differentiation along granulocytic, erythroid, megakaryocytic, monocytic, and B lymphoid pathways.3-11

In eight younger patients with ANLL, however, the expression of differentiation of the involved progenitor cells appeared limited to the granulocyte-macrophage pathway.2,9

To our knowledge, there are no reports of G6PD studies in ALL at the time of presentation. We therefore studied girls with ALL who were heterozygous for G6PD to determine whether their disease was clonal at the time of presentation, whether it involved progenitors with restricted or multipotential expression of differentiation, and whether the G6PD type in malignant cells at relapse was similar to that at diagnosis.

PATIENTS AND METHODS

Clinical features, immunophenotypes, and cytogenetic results are summarized below and in Table 1. Only one child (patient No. 5) had a thymic mass. By French–American–British (FAB)12 criteria, lymphoblast morphology was L-1 in 12 patients, L-2 in three, and L-3 in one (patient No. 12). Patient No. 11 initially had diffuse, malignant non-Hodgkin’s lymphoma without marrow involvement but later had a leukemic conversion. The slides of two patients were not available for review of morphology. Blast cells from 13 of the 17 patients tested were positive for periodic acid-Schiff (PAS), and cells of all 11 of the patients tested for terminal transferase were positive. Sudan black and peroxidase stains, done on marrow samples from all patients except No. 11, were negative. By criteria previously reported13-15 six patients had HLD-DR+, CALLA+ (common) ALL; four had HLA-DR+, CALLA- ALL; two had cytoplasmic immunoglobulin (lg) + pre-B cell ALL; and four had T cell ALL. The cells of two other patients failed to form rosettes with sheep red blood cells, but because other immunophenotyping tests were not performed, the disease in these patients was designated E-.

These two patients, and the one patient whose cells were not tested for surface markers, are considered to have ALL of undefined immunophenotype.

Karyotypes are described in Table 1. The cytogenetic studies from patients No. 1, 3 through 5, 8, 10, 12, 15, 18, and 19 were performed in Memphis by a direct marrow technique developed for ALL.17 Chromosome studies of cells from other patients were performed in Chicago (patients No. 6 and 14) or Seattle (patients No. 13 and 16). Karyotypic abnormalities were found in marrow cells of ten of the 14 patients. None of the patients had a Philadelphia chromosome and none had the t(8;14), t(11;14), or t(1;19) translocation, changes that have been associated with specific immunophenotypes of ALL,16 or a breakpoint on chromosome 12 at band p12. Cells from patient No. 8 were studied before banding techniques were routinely established by the laboratory; however, the size and appearance of the marker and pattern of the chromosomes appear to fit what would be

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CLONAL DEVELOPMENT OF ALL

Table 1. Clinical and Laboratory Findings at Diagnosis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Phenotype</th>
<th>FAB</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>HLA-DR+, CALLA−</td>
<td>L-1</td>
<td>46,XX,t(11;1)(p23;q25)</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>HLA-DR+, CALLA−</td>
<td>L-1</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>HLA-DR+, CALLA+</td>
<td>L-1</td>
<td>46,XX,−21,+mar,2p+,7q+ / 47,XX,+19,−21,+mar,2p+,7q+</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>E−</td>
<td>L-1</td>
<td>46,XX,+7,+19,+mar,1,+mar,2</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>HLA-DR+, CALLA+</td>
<td>L-1</td>
<td>Pseudodiploid with marker chromosomes</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>Pre-B cell</td>
<td>L-2</td>
<td>46,XX/47,XX,+7,+19,+mar,1/</td>
</tr>
<tr>
<td>7</td>
<td>19</td>
<td>T cell</td>
<td>L-1</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>8/12</td>
<td>HLA-DR+, CALLA−</td>
<td>L-1</td>
<td>46,XX,+B,+C,+16,+mar,2p,+7q− /</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>HLA-DR+, CALLA+</td>
<td>L-1</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>T cell</td>
<td>ND</td>
<td>Diploid, not banded</td>
</tr>
<tr>
<td>11</td>
<td>9</td>
<td>HLA-DR+, CALLA+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>HLA-DR+, CALLA−</td>
<td>L-3</td>
<td>46,XX,+16,+1/48 with stemline unidentified</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>ND</td>
<td>L-2</td>
<td>47,XX+p+,+C(8 or 9),6q−,+19p+</td>
</tr>
<tr>
<td>14</td>
<td>6</td>
<td>E−</td>
<td>L-1</td>
<td>43,XX,+X,+4,+17t(1;3)(p36;p21), t(9;20)(p22;q13)</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>HLA-DR+, CALLA+</td>
<td>L-1</td>
<td>48 with stemline unidentified</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>E−</td>
<td>ND</td>
<td>46,XX</td>
</tr>
<tr>
<td>17</td>
<td>5</td>
<td>Pre-B cell</td>
<td>L-1</td>
<td>ND</td>
</tr>
<tr>
<td>18</td>
<td>4</td>
<td>HLA-DR+, CALLA+</td>
<td>L-2</td>
<td>46,XX</td>
</tr>
<tr>
<td>19</td>
<td>13</td>
<td>T cell</td>
<td>L-1</td>
<td>46,XX</td>
</tr>
</tbody>
</table>

Samples from patients No. 1 through 7 were studied for G6PD at the time of diagnosis; those from patients No. 8 through 16 were studied at relapse; and those from patients No. 17 through 19, at both diagnosis and relapse. Laboratory findings are those at diagnosis, except that cytogenetic studies for patients No. 10, 14, and 15 and surface marker studies for patient No. 16 were done only at relapse. E-blasts did not form rosettes with sheep erythrocytes; other surface marker studies were not performed. ND, not done or inadequate study.

RESULTS

The results of hematologic and G6PD studies are summarized in Tables 2 through 4. Each of the 19 patients was considered to be heterozygous for G6PD on the basis of tests of non-leukemic nucleated cells. During remission, all patients manifested both G6PD types in their blood and marrow mononuclear cells (density <1.077 g/cc), granulocytes (dextran-sedimented cells) and platelets, and red blood cells (for the A/B heterozygotes) (Table 2). The ratios of A:B were similar to those found in skin. Similarly, at the time of active disease, morphologically normal hematopoietic cells, such as erythrocytes, granulocytes, and platelets, showed both G6PD types in ratios comparable to remission cells and skin (Tables 3 and 4).

The results of studies at the time of active disease are given in Tables 3 and 4. Cells from patients No. 1 through 7 were evaluated at the time of diagnosis, those from patients No. 8 through 16, at relapse; and those from patients No. 17 through 19, at both diagnosis and relapse. Lymphoblasts from all patients studied at the time of active marrow disease displayed only one enzyme type. For example, the marrow blasts of patient No. 1 showed only G6PD type A and those of patient No. 2, only G6PD type B.

Mixed cell populations containing a predominance of lymphoblasts and a minor component of morphologically normal cells showed a preponderance of one G6PD enzyme. For example, mononuclear cells from the blood of patient No. 5 were predominantly lymphoblasts and showed 90% G6PD type A. The finding of a minor component of type B enzyme activity is consistent with an admixture of lymphoblasts with normal cells (12% of the patient's circulating mononuclear cells were not morphologically classified as leukemic). Likewise, the blood mononuclear cells of patient No. 14 had a predominance of G6PD type A in studies at relapse, consistent with the blood lymphoblast counts.

To determine the significance of the minor component of such cell populations, light-density marrow cells were stained with an anti-CALLA monoclonal antibody, isolated by fluorescence-activated cell sorting, and then tested for...
G6PD. Unsorted light-density marrow cells of patients No. 3 and 6 showed predominantly one G6PD, but a minor enzyme component was also present; sorted CALLA-positive cells exhibited only a single enzyme (Table 3). Similarly, the sorted CALLA-positive marrow cells of patients No. 18 showed only one G6PD.

G6PD was studied in cells from extramedullary sites of three patients. Patient No. 7 failed to achieve remission after five-drug induction therapy. A lymph node removed after six weeks of therapy was heavily infiltrated by lymphoblasts. G6PD tests done on a cell suspension from the node showed 15% type A and 85% type B, compared with blood mononuclear cells at presentation that were 86% lymphoblasts and exhibited only G6PD type B. Patient No. 15 had an extramedullary ovarian relapse. The ovarian tumor and Ficoll-Hypaque-separated cells from the ascitic fluid displayed only G6PD type A. At autopsy, leukemic cells were found in the thymus and posterior mediastinum and showed only G6PD type A. Patient No. 16 had a central nervous system relapse, and blasts from cerebrospinal fluid manifested only type A. At autopsy, five months later, samples of a liver nodule, inguinal node, and retroperitoneal mass composed of leukemic blasts also showed only G6PD type A, in contrast to normal tissues, which showed 50% G6PD type A and 50% G6PD type B.

Cells from three patients were studied at diagnosis and relapse. In each case the leukemic lymphoblasts were of the same G6PD type at both times of study. Patient No. 17 was initially diagnosed in June 1981 and had a hematologic relapse in February 1984, after 30 months of continuous complete remission. Marrow mononuclear cells showed only G6PD type A at both times. Patient No. 18 relapsed after 31 months of continuous complete remission and had a second hematologic relapse nine months later. CALLA-positive cells obtained by cell sorting during the second relapse showed only G6PD type B, similar to results for Ficoll-Hypaque-separated blood and marrow cells obtained at the time of diagnosis. Patient No. 19 developed recurrent disease after 12 months of continuous complete remission, and her blasts manifested G6PD type A at both diagnosis and relapse.

DISCUSSION

In previous studies reported in abstract form, we found only one G6PD type in leukemic blasts from four patients.

### Table 3. Percentage of G6PD Type A at Times of Active Disease

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease Status</th>
<th>Marrow MNC</th>
<th>Gran. RBC</th>
<th>Blood MNC</th>
<th>Gran. PIt.</th>
<th>RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diagnosis 100</td>
<td>ND</td>
<td>50*</td>
<td>100</td>
<td>ND</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>Diagnosis 0</td>
<td>ND</td>
<td>0</td>
<td>20</td>
<td>20</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>Diagnosis 15:0†</td>
<td>20</td>
<td>50</td>
<td>40</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Diagnosis 0†</td>
<td>ND</td>
<td>0*</td>
<td>25</td>
<td>25</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Diagnosis ND</td>
<td>ND</td>
<td>0</td>
<td>90</td>
<td>ND</td>
<td>0*</td>
</tr>
<tr>
<td>6</td>
<td>Diagnosis 95:100†</td>
<td>ND</td>
<td>0</td>
<td>95</td>
<td>35</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>Diagnosis ND</td>
<td>ND</td>
<td>0</td>
<td>ND</td>
<td>25</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>2nd HR 100</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>9</td>
<td>1st HR 0</td>
<td>ND</td>
<td>0</td>
<td>5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>2nd HR 0</td>
<td>20</td>
<td>0</td>
<td>25</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>11</td>
<td>3rd HR 0</td>
<td>15</td>
<td>0</td>
<td>10</td>
<td>10</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>1st HR 100</td>
<td>ND</td>
<td>10</td>
<td>50</td>
<td>50</td>
<td>ND</td>
</tr>
<tr>
<td>13</td>
<td>2nd HR 0</td>
<td>ND</td>
<td>45</td>
<td>ND</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>1st HR ND</td>
<td>ND</td>
<td>ND</td>
<td>80</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>15</td>
<td>Ovarian relapse: ovarian tumor and ascitic fluid cells—type A only</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>16</td>
<td>Cerebrospinal fluid blasts: type A only</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>17</td>
<td>Diagnosis 100</td>
<td>ND</td>
<td>50</td>
<td>100</td>
<td>ND</td>
<td>50</td>
</tr>
<tr>
<td>18</td>
<td>Diagnosis 0</td>
<td>ND</td>
<td>0*</td>
<td>25</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>Diagnosis ND</td>
<td>ND</td>
<td>ND</td>
<td>85</td>
<td>ND</td>
<td>35</td>
</tr>
</tbody>
</table>

RBC, red blood cells; MNC, Ficoll-Hypaque-separated light-density cells, <1.077 g/cm^3; Buffy coat cells (patients No. 1 and 19); Gran., dextran-sedimented cells unless otherwise noted; PIt., platelets; HR, hematologic relapse; ND, not done.

*After transfusion.
†Cells sorted after incubation with anti-CALLA monoclonal antibody.

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**On the basis of G6PD findings in red cells during remission, patients No. 1, 3, 8, 11 through 13, 16, 17, and 19 were considered to be heterozygous for Gd^A_ and Gd^A_. Patients No. 2, 4 through 7, 9, 10, 15, and 18 were presumed to be heterozygous for Gd^A_ and Gd^A_. In the patients with Gd^A_, the failure to detect type A—G6PD is not surprising. That enzyme is unstable and not synthesized in mature red cells since they lack nuclei. Patient No. 14 was not studied during remission, but a comparison of findings in granulocytes and erythrocytes at relapse suggested that she was Gd^A_/Gd^A_. For skin, most studies were performed on cells obtained directly from skin biopsies. Exceptions were cells from patients No. 9 and 19, for whom skin fibroblasts in cultures were studied. ND, not done.

*Light-density mononuclear cells, <1.077 g/cc.
†Dextran-sedimented cells, or Buffy-coat cells (patients No. 7 and 10).
‡At time of marrow remission, patient had splenomegaly.
with ALL in relapse.34 We now extend these findings to a larger group of patients with different immunologic types of
ALL who were studied at diagnosis as well as at relapse. In each case the leukemic lymphoblasts showed only one G6PD
type. When the study included blasts from different anatomical
sites or blasts that were collected at different intervals in
the clinical course, the same G6PD type was found.

Lymphoid neoplasias are thought to develop from a single
abnormal progenitor cell capable of expansion by indefinite
self-renewal. Support for the clonal development of lympho-
proliferative disorders has come mainly from cytogenetic,21
Ig gene,23 and G6PD studies of leukemic precursor cells.
Possible exceptions to the usual finding of clonal derivation
are suggested by recent studies of Ig gene configuration.
Kitchingman et al,34 using a Cu heavy-chain Ig gene probe,
identified multiple rearranged bands in eight of 52 cases of
non-T, non-B ALL, which may indicate the presence of more
than the occurrence of two independent clones. Ig heavy-and
light-chain genes are sequentially rearranged during B cell
differentiation,26,27 whereas G6PD markers have no apparent
relationship to postembryonic hematopoietic differentiation.
The G6PD type expressed in each cell is determined early in
embryonic development, before hematopoiesis, and thus
affords a more reliable method for determining the ultimate
clonality of lymphoproliferative disease.

Cells of the three patients who were studied at diagnosis and
relapse had the same G6PD type at both stages of
disease, consistent with involvement of the same leukemic
clon. These patients all relapsed within 31 months of
diagnosis. Most cytogenetic findings in ALL provide support
for the same leukemic clone being present at diagnosis and
relapse.21,28 Williams et al,34 however, recently reported
cytogenetic data indicating the presence of different clones
during diagnosis and relapse in two (4%) of 51 patients with ALL.
None of our patients had such karyotypic findings, although
sidelines consistent with clonal evolution were noted. In
Burkitt’s lymphoma, studies of 40 patients who had early
recurrent disease (within five months of initial presentation)
showed concordant G6PD and Ig phenotypes, consistent with
reemergence of the originally detected malignant clone. On
the other hand, four of 13 patients with late recurrent disease
showed discordant G6PD or Ig types consistent with emer-
gence of malignant clones that were either undetected at
diagnosis or induced after initial treatment.29 However, such
recurrent tumors were still clonally derived. Occasional
reports of leukemic relapse in donor cells after bone marrow
transplantation provide additional support for the idea that
relapses in ALL may not uniformly involve the original
clone.30,31 Additional studies of patients with ALL who are
erozygous for G6PD are needed to define the relationship
between initial and relapsed disease.

Both G6PD types were present in morphologically normal
hematopoietic cells at times of active disease and during
remission, indicating that these populations descended from

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Table 4. Hematologic Studies at Times of Active Disease

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Disease Status</th>
<th>Marrow Lymphoblasts (%)</th>
<th>Blood Leukocyte Count (10^9/L)</th>
<th>Blood Blasts (%)</th>
<th>Blood Myeloid Cells (%)</th>
<th>Other Mononuclear Cells (%)</th>
<th>Platelet Count (10^9/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diagnosis</td>
<td>70</td>
<td>153.0</td>
<td>75</td>
<td>5</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Diagnosis</td>
<td>89</td>
<td>20.7</td>
<td>83</td>
<td>6</td>
<td>11</td>
<td>216</td>
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<tr>
<td>3</td>
<td>Diagnosis</td>
<td>100</td>
<td>4.0</td>
<td>2</td>
<td>31</td>
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<td>186</td>
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<td>97</td>
<td>8.5</td>
<td>6</td>
<td>38</td>
<td>62</td>
<td>84</td>
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<tr>
<td>5</td>
<td>Diagnosis</td>
<td>88</td>
<td>81.7</td>
<td>86</td>
<td>2</td>
<td>12</td>
<td>50</td>
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<tr>
<td>6</td>
<td>Diagnosis</td>
<td>92</td>
<td>72.0</td>
<td>89</td>
<td>11</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>Diagnosis</td>
<td>97</td>
<td>275.0</td>
<td>86</td>
<td>14</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>8</td>
<td>2nd HR*</td>
<td>76</td>
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<td>0</td>
<td>54</td>
<td>46</td>
<td>400</td>
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<td>65</td>
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<td>282</td>
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<td>2.6</td>
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<td>44</td>
<td>120</td>
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<td>2nd HR</td>
<td>100</td>
<td>8.0</td>
<td>18</td>
<td>34</td>
<td>48</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
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<td>ND</td>
<td>11.8</td>
<td>40</td>
<td>25</td>
<td>35</td>
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<tr>
<td>17</td>
<td>Diagnosis (7/81)</td>
<td>93</td>
<td>22.9</td>
<td>95</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>Diagnosis</td>
<td>100</td>
<td>4.6</td>
<td>7</td>
<td>4</td>
<td>89</td>
<td>58</td>
</tr>
<tr>
<td>19</td>
<td>1st HR</td>
<td>90</td>
<td>166.5</td>
<td>82</td>
<td>2</td>
<td>16</td>
<td>10</td>
</tr>
</tbody>
</table>

The results of the studies are based on Wright-stained smears. ND, not done.

*Hematologic relapse.
normal progenitors. This finding contrasts with observations in CML, and in some patients with ANLL, indicating that erythrocytes and platelets may descend from the abnormal clone and hence from a multipotential stem cell.\textsuperscript{5,6,12} The finding that clonality in ALL is limited to morphologically identifiable lymphoblasts is analogous to findings in some cases of ANLL, in which only granulocytic–monocytic differentiation from the leukemic stem cell was found.\textsuperscript{13,14} Our findings indicate that ALL involves a progenitor cell in which expression of differentiation is restricted to lymphoid pathways. Whether this cell is a committed lymphoid progenitor or a pluripotent stem cell in which myeloid differentiation is blocked requires further investigation.

In some density-separated mononuclear cell preparations containing mostly lymphoblasts, both enzyme types were observed. Two explanations for these findings are that the tested cell populations were not homogenous for malignant cells or that the leukemias had multicellular origins. To distinguish between these possibilities, we sorted lymphoblasts that expressed the CALLA marker and found that CALLA-positive cells showed only one G6PD. Similar results were obtained when this technique was used to study leukemic blasts that were a minor component of the mononuclear cell population (patient No. 18). This cell-sorting technique is useful for preparing homogenous cell populations and enables study of G6PD in discrete cell populations associated with specific markers of cell differentiation. Further studies with cell sorting may permit delineation of the differentiation state of leukemic lymphoblast progenitors by relating G6PD to genotypic or phenotypic changes that are associated with cell differentiation.

In summary, the data reported here indicate that (1) ALL is a clonal disease at diagnosis and at relapse; (2) leukemic lymphoblasts from multiple sites are derived from the same clone; (3) the expression of differentiation by the progenitor cell involved in ALL appears to be limited to the lymphoid pathway, either because that cell is a progenitor committed to lymphoid differentiation or because it is a pluripotent stem cell in which myeloid differentiation is blocked.

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Evidence for clonal development of childhood acute lymphoblastic leukemia

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