Determining blast cell biological features at diagnosis of acute lymphoblastic leukemia (ALL) has clinical relevance in the context of cell classification schemes and particular treatment programs. Numerous studies have demonstrated close relationships between the outcome of therapy and blast cell morphology, surface immunophenotype, number of glucocorticoid receptor sites per cell and, more recently, chromosomal abnormalities (see ref 1 for review). Repeated assessment of selected markers at later intervals in the clinical course might be expected to contribute important information for understanding mechanisms of disease recurrence or for planning alternative therapy. Most previous studies have indicated that blast cell phenotypes remain stable over time, with only minor changes occurring in a small proportion of patients. With the availability of comprehensive assays for lymphoid and myeloid cell markers, however, an increasing number of reports have claimed major phenotypic shifts in ALL.

We had the opportunity to study 68 cases of ALL at both diagnosis and relapse to document possible changes in lymphoblast phenotype. The results indicate a relatively high frequency of phenotypic shift in ALL, mainly disappearance with the loss of CALLA. Results of chromosomal analyses in cases with a loss of CALLA implicated several mechanisms in the observed phenotypic changes. In six cases, including each instance of lineage switch, the original karyotype had been replaced by an entirely different abnormal karyotype, suggesting clonal selection or induction of a second malignancy. In another case, the evidence suggested clonal evolution. Our findings demonstrate that sequential phenotypic and cytogenetic studies may yield valuable insights into the mechanisms of leukemic recurrence and may have implications for treatment selection.

MATERIALS AND METHODS

One hundred twenty-seven children with ALL who were admitted to Total Therapy Study X° at St. Jude Children's Research Hospital experienced their first hematologic relapse between September 1980 and October 1985. Sixty-eight patients had adequate bone marrow samples for blast cell immunophenotyping at both diagnosis and relapse and are the subjects of this report. Informed written consent was obtained from the patients, their parents, or both.

Immunologic cell typing. Bone marrow cells were separated on a Ficoll-Hypaque gradient, and only samples containing >85% blasts were assayed. They were considered positive for heat-stable E rosette formation (E) 37 °C if at least 2% of the blast cells formed rosettes. Cell-surface antigens were detected by a standard indirect immunofluorescence assay with monoclonal antibodies representative of the major cluster groups (CD) identified at the First and Second International Workshops on Human Leukocyte Differentiated Antigens. They included J5 (CALLA, or CD-1), HLA-DR, T10, and T cell-specific antigens (T)-T101 (CD-5), T3 (CD-3), and T11 (CD-2). Two investigators independently counted 200 cells in each immunofluorescence assay; the results were judged positive if >40% of the blast cells had surface fluorescence.

Cell-surface immunoglobulin (SIg) was identified by direct fluorescence with fluoresceinated goat anti-human immunoglobulin. Cytoplasmic immunoglobulin (C Ig) was identified by a direct method that used fluoresceinated F(ab')2 goat anti-human χ-chains (Southern Biotechnology, Birmingham, AL); positive cases were those in which ≥10% blast cells contained C Ig. Depending on their pattern of reactivity, the samples were classified phenotypically as common ALL (CALLA+, HLA-DR+, E+, T+, C1g+, SIg+), pre-B cell (CALLA+, HLA-DR+, C1g+, SIg+, T+), or CALLA- (HLA-DR+, CALLA-, C1g+, SIg+, T+). Terminal deoxynucleotidyl transferase (TdT) was identified by use of indirect immunofluorescence. Cells from selected patients were examined for myeloid-associated surface antigens: My-1 (X-Hapten, CD-15), M-CS-F1 (CDw-13), SF1 (CDw-14), Mol (CD-1), and SI-D1 (gp 50).

Cytotoxic chemical studies. Bone marrow smears were stained by standard techniques including periodic acid-Schiff reagent, Sudan black B, myeloperoxidase, naphthol AS-D chloroacetate esterase, and α-naphthyl butyrate esterase. The presence of >3% positive
blast cells was considered a positive result. Morphologic classification followed French-American-British (FAB) conventions.

Cytogenetic studies. Bone marrow samples were prepared by a direct technique developed for ALL and metaphases were G-banded by a modified Trypsin method.

RESULTS

Of the 68 patients who were studied sequentially, 39 had common ALL at diagnosis, 16 had pre-B (12 CALLA +), 8 had CALLA −, and 5 had T cell ALL. Although the number of cell marker studies performed for each sample varied according to the number of leukemic blast cells available, tests for CALLA and HLA-DR were performed in every case.

Shifts in antigen expression at relapse are shown in Table 1. A frequent alteration involved the loss of CALLA in five cases of common ALL and in three of pre-B ALL. In no instance did blast cells acquire CALLA after being negative for the antigen at diagnosis. The HLA-DR antigen was lost in 1 pre-B and 2 common ALL cases, but was acquired in 1 of 3 common and 1 of 3 pre-B cases that originally lacked this marker. TdT activity was evident at diagnosis in all but 2 of the cases tested (both common ALL), decreasing to negligible levels in 3 of 10 cases of common ALL and 3 of 10 pre-B cases that were examined at relapse. TdT activity remained positive in the three CALLA − cases tested. The T10 antigen was detected at diagnosis on blast cells in 10 of the 17 non-T cases that were tested, appearing at relapse in 6 of the 7 T10-negative cases.

Loss of CALLA was associated with conversion to acute nonlymphoblastic leukemia (ANLL) in two cases that initially had common ALL and in one patient with CALLA − pre-B leukemia. The immunologic and cytochemical studies of blast cells from these three cases are summarized in Table 2. Besides the loss of CALLA, both tested cases lacked TdT activity, and Clg was no longer detectable in the case initially classified as pre-B. Notably, myeloid-associated surface antigens were present at diagnosis on the blast cells from patient 1. At diagnosis, the leukemic cells from all three patients met stringent morphologic criteria for ALL and lacked cytochemical evidence of myeloid or monocytic features. When examined at relapse, the cells reacted with myeloid-associated cytochemical stains, and Auer rods were present in case 4.

The results of sequential cytogenic studies in the eight cases showing a loss of CALLA are presented in Table 3. Evidence for a different leukemic clone at relapse was obtained in six of the cases. The original blast cell karyotype of cases that later converted to ANLL was replaced by an entirely different karyotype. At the time of lineage switch, each case had a pseudodiploid karyotype and a different translocation involving the long arm of chromosome 11 at band q23 (11q23). The other three cases, which had converted from common to CALLA − ALL, also had different pseudodiploid karyotypes at relapse, characterized by a translocation involving 11q23 in the two with complete banding study. Of the remaining two cases, one (patient 13) showed evidence of marked clonal evolution, and the other (patient 12) retained the apparent constitutional abnormality.

DISCUSSION

Seventeen (25%) of the 68 patients in this series had blast cell phenotypic shifts at relapse. Loss of the common ALL antigen was a frequent change, occurring in 16% of cases that were clearly CALLA + at diagnosis. Greaves and colleagues also demonstrated a loss of CALLA in similar
Table 2. Laboratory Findings in Cases of Lineage Switch

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>LS</th>
<th>Diagnosis</th>
<th>LS</th>
<th>Diagnosis</th>
<th>LS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunological markers†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Common ALL antigen</td>
<td>96</td>
<td>6</td>
<td>76</td>
<td>3</td>
<td>70</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>86</td>
<td>84</td>
<td>35</td>
<td>52</td>
<td>78</td>
</tr>
<tr>
<td>E rosette formation</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>0</td>
</tr>
<tr>
<td>T101</td>
<td>4</td>
<td>19</td>
<td>ND</td>
<td>ND</td>
<td>91</td>
</tr>
<tr>
<td>Cytoplasmic immunoglobulin</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>84</td>
</tr>
<tr>
<td>Terminal transferase</td>
<td>96</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>My-1</td>
<td>68</td>
<td>22</td>
<td>ND</td>
<td>ND</td>
<td>60</td>
</tr>
<tr>
<td>MCS-2</td>
<td>44</td>
<td>66</td>
<td>ND</td>
<td>ND</td>
<td>55</td>
</tr>
<tr>
<td>Sf-1</td>
<td>39</td>
<td>38</td>
<td>ND</td>
<td>28</td>
<td>ND</td>
</tr>
<tr>
<td>Mol</td>
<td>48</td>
<td>26</td>
<td>ND</td>
<td>21</td>
<td>ND</td>
</tr>
<tr>
<td>SJ-D1</td>
<td>54</td>
<td>64</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cytochemical stains†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sudan black B</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>−</td>
<td>5</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Chloroacetate esterase</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>ND</td>
<td>−</td>
</tr>
<tr>
<td>a-Naphthyl butyrate esterase</td>
<td>−</td>
<td>56</td>
<td>−</td>
<td>ND</td>
<td>−</td>
</tr>
</tbody>
</table>

LS, lineage switch; ALL, acute lymphoblastic leukemia; ND, not done; FAB, French-American-British; +, positive; −, negative.
†Presence of myeloid-associated surface antigens at diagnosis.
†Results given as percentages of positive cells.
‡Auer rods present.

proportions of cases studied at diagnosis and relapse; however, there is little information regarding the significance of changes in CALLA expression at relapse.

Of the 8 cases that converted from CALLA⁺ to CALLA⁻, 3 showed lineage switch to ANLL, 2 were refractory to retreatment, and 3 responded to therapy; their reinduced remissions were short, however (2, 5, and 10 months, respectively). In contrast, 12 of the 43 patients who retained CALLA at relapse remain in second remission for 7+ to 56+ months (median, 23+ months). Aside from the length

Table 3. Cytogenetic Findings in Cases With a Loss of CALLA

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Time to Relapse (mo)</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
<th>At Diagnosis</th>
<th>Immunophenotype</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>Common ALL</td>
<td>47,XY,+9</td>
<td>ANLL (M5)</td>
<td>46,XY,−18,+der(18)(p11;3,7),t(9;11)(p21;q23)/46,XY.t(9;11)(p21;q23)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>Common ALL</td>
<td>46,XX,−20,t(2;16)(p13;p13),45,XX,−20,+ +,mar,t(2;16)(p13;p13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>Common ALL</td>
<td>46,XX,−19,+der(19)(q13;p33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>69</td>
<td>Common ALL</td>
<td>54,XY,+X,+5,+6,+10,+11,+D,+21,del(7)(q22)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>Common ALL</td>
<td>53,XY,+X,+6,+9,+15,+17,+21,mar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>36</td>
<td>Pre-B ALL</td>
<td>46,XY,−12,+der(12)(q13;3),t(12;13)(q12;13)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>9</td>
<td>Pre-B ALL</td>
<td>46,XY,t(1;19)(q23;p13;3),t(19;14)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>27</td>
<td>Pre-B ALL</td>
<td>46,XY,−13,+der(13)(q13;3),t(13;q34,?),t(11;19)(q23;p13;3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CALLA, common ALL antigen; ALL, acute lymphoblastic leukemia; ANLL, acute nonlymphoblastic leukemia.
*Band preparation was inadequate for complete interpretation. The marker is a metacentric chromosome of group A.
†The inv(9) was interpreted as a constitutional abnormality that occurs commonly in the general population.
of a patient’s initial hematologic remission, there are no reliable predictors of outcome following a bone marrow relapse. Our data suggest that loss of CALLA is a poor prognostic sign in such cases. Despite the relatively long initial periods of hematologic remission in several of our CALLA⁺→CALLA⁻ patients (Table 3), ultimate outcome in this group has been poor. The apparent adverse prognostic effect of CALLA loss from leukemic cells should be interpreted with some caution, however, as our study population was relatively small, had been treated in several different protocols for relapsed leukemia, and had variable durations of initial hematologic remission.

Acquisition or loss of the HLA-DR antigen, noted in five of our patients, has been observed in previous studies. Loss of blast cell TdT activity was also a common finding. In two of the six patients tested, decreased TdT activity at relapse was associated with lineage switch. Additional studies are needed to determine the significance of this alteration. Blast cells from 10 of 17 patients with non-T ALL showed T10 positivity at diagnosis; in the other 6 cases, the antigen was acquired at relapse, confirming reports that T10 is not restricted to cells of T lineage.

The molecular basis for aberrant regulation of lineage-specific differentiation antigens in ALL is unknown; however, the karyotypes available for cases showing a loss of CALLA (Table 3) suggest several mechanisms of phenotypic shift at the chromosomal level. We think it remarkable that in cases 1 through 5 and 11, the original karyotype was replaced by an entirely different abnormal karyotype, suggesting induction of a second malignancy or perhaps selection and overgrowth of an independent drug-resistant clone that could not be detected at diagnosis. We favor the first explanation because of the relatively long intervals to relapse, 2 to 5 years, in the three cases that converted to ANLL, and the lack of evidence for separate populations of myeloid and lymphoid leukemic cells coexisting in the same patient at diagnosis. At relapse, each of the cases with complete banding analyses possessed a chromosomal translocation involving 11q23, a region commonly affected by cytogenetic changes in the myelomonocytic subtype of ANLL, CALLA⁺ ALL, and leukemia with mixed-lineage expression. It has been suggested that pluripotent stem cells are transformed in leukemia with 11q23 involvement, and that phenotypic expression is influenced by genes from the reciprocally translocated chromosome. Thus, loss of CALLA in these cases may be related to malignant transformation of a pluripotent stem cell after eradication of the original B cell-precursor and pre-B stem line by chemotherapy. The c-ets-1 oncogene was mapped to the 11q23 region and may be involved in the pathogenesis of acute leukemias, with a translocation affecting this locus. Indeed, Rovigatti and colleagues recently demonstrated amplification and rearrangement of this oncogene in a case of acute myelomonocytic leukemia and a case of small lymphocytic cell lymphoma with 11q23 involvement. Case 12 cannot be interpreted because of failure to demonstrate a malignant stem line at relapse. Case 13 represents the only instance in which karyotypic findings at diagnosis were retained in the reemergent blast cells (clonal evolution).

Although not observed in the T cell cases in this series, lineage switch has occurred in childhood leukemia of thymic origin. Stass and co-workers, for example, showed conversion to ANLL in five cases that were initially classified as T cell ALL. Early recognition of lineage switch may be helpful in selecting an effective plan of retreatment. Each of the three patients who had undergone conversion to myeloid leukemia in this study achieved a second remission with ANLL-directed induction therapy. Finally, monoclonal antibodies with complement or those conjugated to ricin have been used to purge bone marrow before autologous transplantation. For such therapeutic approaches, it is essential to have an accurate profile of antigen expression at relapse.

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REFERENCES

12. Royston I, Majda JA, Baird SM, Meserve BL, Griffiths JC: Human T-cell antigens defined by monoclonal antibodies: The
65,000-dalton antigen of T-cells (T65) is also found on chronic lymphocytic leukemia cells bearing surface immunoglobulin. J Immunol 125:725, 1980


35. Foon KA, Schroff RW, Gale RP: Surface markers on leukemia and lymphoma cells: Recent advances. Blood 60:1, 1982
Shifts in blast cell phenotype and karyotype at relapse of childhood lymphoblastic leukemia [published erratum appears in Blood 1987 Mar;69(3):996]

CH Pui, SC Raimondi, FG Behm, J Ochs, WL Furman, NJ Bunin, RC Ribeiro, PA Tinsley and J Mirro