CONCISE REPORT

Occurrence of the Common Acute Lymphoblastic Leukemia Antigen on Blast Cells of a Patient with Chronic Myelomonocytic Leukemia in Non-Lymphoid Blastic Phase

By Volker Gressler, Manfred Garbrecht, and Dieter K. Hossfeld

Leukemias showing a conspicuous lymphoid phenotype, i.e., those that are HLA-DR positive, common acute lymphoblastic antigen (cALLA) positive, terminal deoxynucleotidyl transferase (TdT) negative, as well as myeloperoxidase positive (MPO), could be considered so-called mixed leukemias. Leukemias with biphenotypic blasts have to be distinguished from cases comprising two separate subpopulations that express different lineage-associated characteristic. By use of a simple new method (Immunogold Staining) we examined a case of chronic myelomonocytic leukemia in blastic phase and demonstrated simultaneous staining for MPO/alpha-naphthyl-esterase and expression of the HLA-DR-positive, cALLA-positive, and TdT-negative phenotype. The cALL antigen was detected only on mono- and myelo-monoblasts; its expression was inversely related to the MPO positivity, and it disappeared together with these types of blasts after chemotherapy. On the basis of our findings it remains obscure whether the cALL antigen at the initial presentation was due to the immature monocytic features of the leukemic cells or disclosed an additional lymphoid differentiation pattern of the blasts.

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marker analysis shows the majority of cells belonging to the granulocytic and myelomonocytic lineage (MA 66%, Ia 26%). During relapse, the percentage of blasts in the differential blood count is 35; immunofluorescence and immunogold staining for cALL is now completely negative.

**DISCUSSION**

There are some reports suggesting the occurrence of distinct populations of blasts with lymphoid and myeloid phenotypes in acute leukemia. Occasionally, leukemic cells show lymphoid and myeloid characteristics simultaneously. These findings are based on the assumption that MPO positivity signals myeloid and TdT positivity signals lymphoid differentiation pathways. Yet recent studies revealed that the presence of TdT activity has to be interpreted as an early state of maturation rather than a special differentiation lineage. Pui et al reported three children with acute leukemia, at least one of whom showed a wide overlap in the percentage of blasts expressing an unusual lymphoid (Ia+, cALL+, TdT-) and myeloid (MPO+) phenotype providing some evidence of a so-called mixed leukemia.

To our knowledge, the case described here seems to be the first one that demonstrates MPO and cALL antigen on the same cell. The simultaneous cytologic, cytochemical, and immunologic characterization of leukemic cells by the immunogold staining method excludes the existence of separate subpopulations with different lineage associated features. On the other hand, the degree of cALLA positivity of blasts could be shown to be inversely related to MPO staining of these cells. Strongly cALLA-positive blasts appeared cytologically as monoblasts, reacted with moAbs to monocytic antigens (M 5, MY 4) and did not stain for MPO. This type of cell disappeared after chemotherapy and a population of more mature, cALLA-negative cells emerged.

Our findings suggest two possible explanations. First, the blast cells show an aberrant expression of monocytic and myelogenous features; the cALLA has to be regarded as an antigen associated with an early stage of monocytic differentiation. A second possibility is that the leukemic cells of our patient are pluripotent stem cells showing a varying accentuation of lymphoid, myeloid, and monocytic differentiation characteristics. The latter explanation would be supported by the detection of MPO+, cALLA+, TdT-, monocytic antigens bearing cells in normal bone marrow. For that purpose, further studies on a larger number of normal bone marrow cells have to be carried out.

**RESULTS**

Cytologic and immunologic findings are listed in Tables 1 and 2. Before the start of chemotherapy, immunologic typing shows that the majority of cells are positive for myelomonocytic and monocytic markers (MY 7, MY 9, MO 1, respectively, M 5, MY 4, and MO 2). The TdT reaction as well as markers of the B lineage (B 1, B 2, B 4) are negative (not listed in Table 2). The Ia-like antigen, indicating either myelomonocytic or monocytic differentiation, is found on 56% of the mononuclear cells (MNC).

Surprisingly, 72% of the cells bind to the moAbs reacting with the cALL antigen. Unspecific binding was excluded by addition of human-AB serum. Identical results are obtained utilizing two different moAbs recognizing the cALL antigen (J 5, cALLA). By the immunogold staining method, the cALL antigen is only detected on the type II blasts according to the FAB classification. The degree of expression on myelomonocytic leukemic cells and myelomonoblasts is inversely related to the MPO positivity of these cells. The Ia-like antigen shows a similar distribution, yet is lacking on type I blasts. The quantitative analysis reveals a good correlation between the immunofluorescence and immunogold staining method with a deviation within a range of 4%. After chemotherapy, the bone marrow specimen shows a decrease of blasts to 12%. The cALL antigen disappears. In accordance with the cytologic findings, the immunologic

### Table 1. Cytological Results

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Blast Phase</th>
<th>Partial Remission</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts</td>
<td>64 (53)</td>
<td>12 (--)</td>
<td>(35)</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>10 (--)</td>
<td>1 (--)</td>
<td>(--)</td>
</tr>
<tr>
<td>Myelocytes</td>
<td>1 (--)</td>
<td>(--)</td>
<td>(--)</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>(--)</td>
<td>(--)</td>
<td>(--)</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>8 (13)</td>
<td>24 (60)</td>
<td>(50)</td>
</tr>
<tr>
<td>Monocytic Cells</td>
<td>13 (25)</td>
<td>57 (10)</td>
<td>(7)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3 (8)</td>
<td>5 (30)</td>
<td>(8)</td>
</tr>
<tr>
<td>Normoblasts</td>
<td>1 (--)</td>
<td>1 (--)</td>
<td>(--)</td>
</tr>
</tbody>
</table>

Evaluation of bone marrow specimens and (1) differential blood counts in various disease states.

Finally, the slides are dehydrated and mounted. Analysis is done by using combined epitransillumination microscopy (C. Zeiss, Oberkochen, W Germany). The immunogold staining method has been developed and described in detail by Geoghegan et al and DeWaele et al. The method was modified and adapted for the typing of leukemic cells.

### Table 2. Immunologic Findings

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Ia</th>
<th>J 5</th>
<th>M 5</th>
<th>MY 4</th>
<th>MO 1</th>
<th>MO 2</th>
<th>M 1</th>
<th>MY 7</th>
<th>MY 9</th>
<th>MA</th>
<th>T 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blastic phase</td>
<td>56+/++</td>
<td>72+/++</td>
<td>20+/+++</td>
<td>44+</td>
<td>56+</td>
<td>56 (+)</td>
<td>66+/+++</td>
<td>74+</td>
<td>92+</td>
<td>(*)</td>
<td>38 (+)</td>
</tr>
<tr>
<td>Partial remission</td>
<td>26+/-</td>
<td>neg</td>
<td>4+/-</td>
<td>5+/-</td>
<td>58+</td>
<td>(*)</td>
<td>(*)</td>
<td>60 (+)</td>
<td>84 (+)</td>
<td>66+</td>
<td>5+</td>
</tr>
<tr>
<td>Relapse</td>
<td>40+/-</td>
<td>neg</td>
<td>15+</td>
<td>25+</td>
<td>9+/+</td>
<td>60+/++</td>
<td>(*)</td>
<td>95+</td>
<td>98+</td>
<td>(*)</td>
<td>(*)</td>
</tr>
</tbody>
</table>

Bone marrow cells; numbers indicate percentage of positive mononuclear cells. To quantitate results obtained by immunofluorescence analysis an intensity scale ranging from weakly positive (+) to strongly positive (+++) was used.

(*)Not done.
REFERENCES


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