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, ie., 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 characteristics. 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 mononcytic features of the leukemic cells or disclosed an additional lymphoid differentiation pattern of the blasts.

The reaction of antiserum with the cALL antigen of leukemic cells was first reported by Greaves et al., and further investigations revealed that the CALL antigen is not specific for "non-T-non-B" lymphoblastic leukemias; cALLA was shown in some high-grade lymphomas, especially Burkitt's lymphoma, normal "lymphoid" cells in regenerating bone marrow, and blasts of patients with chronic myeloid leukemia in lymphoid transformation. The latter authors, surprisingly, found the CALL antigen in four out of 86 cases of acute myeloid leukemia (AML). The observation of HLA-DR positive, TdT negative, yet MPO positive leukemias raises the question of whether these characteristics are displayed simultaneously by individual blasts or by two different populations of leukemic cells. This is a report of a patient with chronic myelomonocytic leukemia (CMML) who developed a non-lymphoid blastic phase. Using a new dual staining method these blasts were found to be cALLA positive and to exhibit myelomonocytic features.

MATERIALS AND METHODS

Patient. The 73-year-old man was first noted to have an abnormal peripheral blood picture in July 1983. At this time he presented with anemia (hemoglobin 8.8 g/dL) and leucoysis (WBC 15.0 x 10^9/L). Peripheral blood differential and bone marrow aspirate were compatible with the diagnosis of CMML. The index of leukocyte alkaline phosphatase (LAP) was normal. Chromosome analysis of the bone marrow cells revealed a normal karyotype. Apart from blood transfusions no therapy was given. The patient was referred to us in October 1984. On physical examination he had petechiae and livid skin changes of the lower extremities that were shown to be due to infiltration by myelomonocytic cells. Lymph nodes, liver, and spleen were not enlarged. Hemoglobin was 9.7 d/dL, WBC 30.6 x 10^9/L, and platelets 28.0 x 10^9/L. The differential blood count revealed 53% blasts, a third of which had monocytic nuclear features. The percentage of blasts in the bone marrow was 64% and 80% of them exhibited a weakly to strongly positive peroxidase reaction. Alpha-naphthyl acetate esterase (ANAE) was strongly positive in 7% of blasts, weakly positive in 53%, and negative in 40% of blasts. Lysozyme in serum and urine was increased to 163 mg/L (normal, 3 to 9) and 1,600 mg/L (normal, 0 to 3), respectively. Lactate dehydrogenase was 3,980 U/L (normal > 240). Chromosome analysis of bone marrow cells again demonstrated a normal karyotype. The patient was diagnosed to have CMML in blastic phase. He received low-dose cytarabino-side therapy (2 x 18 mg sc for 21 days) and entered a partial remission of short duration. On relapse, chemotherapy with VP 16-213 failed to improve his situation, and he died in December 1984.

Immunofluorescence/Immunogold Staining. Leukemic cells were obtained by bone marrow aspiration and from heparinized samples of peripheral blood. Cytologic (Papenheim Staining, standard technique), cytochemical (MPO, ANAE, and periodic acid Schiff-reaction), and immunologic examinations were performed during blastic phase, in partial remission, and in relapse. For immunologic analysis, mononuclear cells were isolated by Ficoll-Hypaque density sedimentation. A panel of commercially available monoclonal antibodies (moAb) were used: HLA-DR (Ia-like) antibody, J 5, MY 4, MO 1, MO 2, MY 7, MY 9, B 1, B 4 (Coulter Counter Laboratories, Hialeah, USA), T 11, M 5 (Ortho Diagnostics, Raritan, USA), MA, cALLA (New England Nuclear, Boston), and TdT reagent (Bethesda Research Laboratories, Neu Isenburg, W Germany). A standard indirect immunofluorescence assay was used. Briefly, 10^6 mononuclear cells are incubated with an adequate dilution of moAb for 30 minutes on ice. Dilution buffer contains fetal calf serum (FCS), human AB-serum, and sodium acid. The cells are then washed three times with phosphate buffered saline (PBS). A second antibody (goat-anti-mouse-IgG(Fab)2-FITC) is added and incubated for another 30 minutes on ice. After three washes, the cells are examined with the fluorescence microscope (C. Zeiss, Oberkoch- en, W. Germany). For the immunogold staining, the cells are incubated with the first moAb as described, but at room temperature. As a second antibody the GAMG3O (goat-anti-mouse-Isotypes) is bound to colloidal gold particles of about 30 nm size) is used. The GAMG3O reagent was purchased from Fa Janssen, Beersel, Belgium. After washing three times the cells are fixed with 0.01% glutaraldehyde and cytocoentrifugation preparations (4 minutes at 250 g) are made. The slides are fixed in 4% formol ethanol. For the peroxidase reaction, the slides are immersed in 3.3 diaminobenzidine reagent for 40 minutes, rinsed in H2O, and drained. Afterwards, the counterstaining is done with Mayer's Haemalaun for ten minutes.

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Results

Cytologic and immunologic findings are listed in Tables 1 and 2. Before the start of chemotherapy, immunologic typing showed 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 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 cALLA 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. The latter explanation would be supported by the detection of MPO+, cALLA+, 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.

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**Table 1. Cytological Results**

<table>
<thead>
<tr>
<th>Disease State</th>
<th>Blastic 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>1 (--)</td>
<td>(--)</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td>-- (1)</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 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. The method was modified and adapted for the typing of leukemic cells.

**Table 2. Immunologic Findings**

<table>
<thead>
<tr>
<th>Surface Marker Analysis</th>
<th>Blastic phase of CMML</th>
<th>Partial remission</th>
<th>Relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia</td>
<td>56 (+)</td>
<td>44 (+)</td>
<td>5 (+)</td>
</tr>
<tr>
<td>J 5</td>
<td>72 (+)</td>
<td>4 (+)</td>
<td>5 (+)</td>
</tr>
<tr>
<td>M 5</td>
<td>20 (+)</td>
<td>5 (+)</td>
<td>5 (+)</td>
</tr>
<tr>
<td>MY 4</td>
<td>44 (+)</td>
<td>58 (+)</td>
<td>92 (+)</td>
</tr>
<tr>
<td>MO 1</td>
<td>56 (+)</td>
<td>66 (+)</td>
<td>74 (+)</td>
</tr>
<tr>
<td>MO 2</td>
<td>56 (+)</td>
<td>66 (+)</td>
<td>92 (+)</td>
</tr>
<tr>
<td>M 1</td>
<td>74 (+)</td>
<td>92 (+)</td>
<td>38 (+)</td>
</tr>
<tr>
<td>MY 9</td>
<td>26 (+)</td>
<td>60 (+)</td>
<td>66 (+)</td>
</tr>
<tr>
<td>MA</td>
<td>5 (+)</td>
<td>(*)</td>
<td>(*)</td>
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<tr>
<td>T 11</td>
<td>(*)</td>
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</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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