Erythroid Blast Crisis in Chronic Myelogenous Leukemia

By Marja Ekblom, Georg Borgström, Eeva von Willebrand, Carl G. Gahmberg, Pekka Vuopio, and Leif C. Andersson

Blood or bone marrow specimens from 22 patients with chronic myelogenous leukemia in blast crisis were studied for the surface expression of glycophorin-A, a marker for early erythroid differentiation. The leukemic blasts were stained with rabbit anti-glycophorin-A antiserum. The glycophorin-A molecules detected by the rabbit antiserum were identified by polyacrylamide slab gel electrophoresis of the immunoprecipitates from the membrane lysates of surface-labeled blasts. Blasts expressing surface glycophorin-A were found in 9 of the 22 patients. In 4 patients, almost all blasts were glycophorin-A positive, and in 5 patients, less than half of the blast population expressed glycophorin-A. The present study shows that when glycophorin-A is used as a marker for erythroid blasts, involvement of the erythroid lineage during blast crisis of chronic myelogenous leukemia seems to occur more frequently than previously recognized.

The identification of malignant cells with a maturation arrest at early stages of erythroid differentiation has been difficult because of the lack of specific markers. In a few cases, intracellular fetal hemoglobin has been found.\textsuperscript{1} The onset of hemoglobin synthesis is, however, a relatively late event during normal erythropoiesis. It is reasonable to assume that malignant blasts in leukemias mostly represent earlier stages of differentiation.

Glycophorin-A (GP-A), which is the major membrane sialoglycoprotein of human red cells and their precursors, is a specific and early marker for the normal erythroid lineage.\textsuperscript{2} In the normal erythroid maturation sequence, it is detectable on the surface of the earliest morphologically recognizable erythroid precursors, the proerythroblasts.\textsuperscript{3}

There are reports indicating that glycophorin-A may be useful for recognition of acute leukemias with an early erythroid phenotype. In approximately 10% of adult acute leukemias the blasts that are classified as lymphoid or undifferentiated myeloid by morphology and cytochemistry express glycophorin-A on their surface membrane.\textsuperscript{1}

Karyotypic and enzyme analyses in the chronic phase of chronic myelogenous leukemia (CML) have shown that the malignant clone may include the erythroid lineage.\textsuperscript{4,5} Erythroid involvement in the acute phase has not, however, been routinely diagnosed. We report that blasts with surface expression of glycophorin-A are found in a significant proportion of patients with chronic myelogenous leukemia in blast crisis.

MATERIALS AND METHODS

Between March 1979 and February 1982, approximately 50 cases of CML in blast crisis have been diagnosed at the III Department of Medicine, Helsinki University Central Hospital. Bone marrow aspirates or peripheral blood samples from 27 of these patients were obtained for surface marker analysis. In 5 cases, immunofluorescent staining for glycophorin-A was not made because of inadequate specimens.

The mononuclear cells were isolated by Ficoll-Isoaque density gradient centrifugation.\textsuperscript{5} Contaminating erythrocytes were lysed by incubation of the cells in 0.83% aqueous solution of Tris-buffered ammonium chloride. Cytocentrifuge preparations of the cells were stained with May-Grünewald-Giemsa and the alpha-naphthyl acetate esterase stains.\textsuperscript{6}

Anti-glycophorin-A antiserum was prepared, as previously described, by immunizing a rabbit with purified glycophorin-A and absorbing the antiserum with membranes of En(a−) red cells, which lack glycophorin-A.\textsuperscript{2}

Rabbit antibodies to HLA-DR (Ia-like) antigen were kindly provided by Prof. P. A. Peterson, Uppsala, Sweden. In some cases, monoclonal anti-DR-antibody (OKIla, Ortho Pharmaceutical Corporation, Raritan, NJ) was used. Tetramethylrhodamine (TRITC) and fluorescein isothiocyanate (FITC) conjugated swine anti-rabbit antiserum and rabbit anti-mouse antiserum, polyclonal rabbit anti-human immunoglobulin, anti-kappa and anti-lambda light chain, anti-μ heavy chain antiserum, and rabbit anti-human factor-VIII-associated antiserum were purchased from Dakopatts, Copenhagen, Denmark. The anti-factor-VIII antiserum did not react with morphologically recognizable erythroid cells in normal bone marrow, but stained the megakaryocytes.

The expression of T-lymphocytic markers was tested by the ability of the cells to form rosettes with AET-treated sheep red cells at 4°C.\textsuperscript{8} The presence of Fc-receptors for IgG was studied by the ability of the cells to form rosettes with rabbit IgG-coated human blood group O cells.

The cells were stained with membrane and intracytoplasmic immunofluorescence. Rosettes with antibody and protein-A-containing Staphylococcus aureus Cowan I strain were made as described.\textsuperscript{7} The cell surface glycoproteins were radiolabeled by the periodate/NaB3H4 method\textsuperscript{9} and immunoprecipitations from the surface-labeled membrane lysates using the protein-A-containing Staphylococcus aureus Cowan I strain were done as described.\textsuperscript{2} Electrophoresis on 8% polyacrylamide slab gels was performed according to Laemmli.\textsuperscript{10} The gels were fixed and treated for fluorography as described.
Table 1. Peripheral Blood and Bone Marrow Findings During Glycophorin-A-Positive Blast Crisis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Hb (g/Liter)</th>
<th>Leukocytes (x 10^9/Liter)</th>
<th>Blasts (%)</th>
<th>Platelets (x 10^9/Liter)</th>
<th>Cellularity (%)</th>
<th>Normoblasts (%)</th>
<th>Myeloid Cells (%)</th>
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<tbody>
<tr>
<td>(1)</td>
<td>76</td>
<td>F</td>
<td>83</td>
<td>4.7</td>
<td>63</td>
<td>432</td>
<td>Normal</td>
<td>75</td>
<td>1</td>
</tr>
<tr>
<td>(2)</td>
<td>38</td>
<td>M</td>
<td>112</td>
<td>12.5</td>
<td>67</td>
<td>89</td>
<td>Increased</td>
<td>68</td>
<td>14</td>
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<td>(3)</td>
<td>67</td>
<td>F</td>
<td>110</td>
<td>19.3</td>
<td>40</td>
<td>99</td>
<td>Increased</td>
<td>69</td>
<td>9</td>
</tr>
<tr>
<td>(4)</td>
<td>60</td>
<td>F</td>
<td>142</td>
<td>17.1</td>
<td>27</td>
<td>375</td>
<td>Increased</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>(5)</td>
<td>34</td>
<td>M</td>
<td>121</td>
<td>4.6</td>
<td>2</td>
<td>37</td>
<td>Normal</td>
<td>58</td>
<td>2</td>
</tr>
<tr>
<td>(6)</td>
<td>16</td>
<td>M</td>
<td>119</td>
<td>37.5</td>
<td>80</td>
<td>49</td>
<td>Increased</td>
<td>83</td>
<td>3</td>
</tr>
<tr>
<td>(7)</td>
<td>39</td>
<td>F</td>
<td>83</td>
<td>75.0</td>
<td>53</td>
<td>50</td>
<td>Increased</td>
<td>93</td>
<td>5</td>
</tr>
<tr>
<td>(8)</td>
<td>60</td>
<td>F</td>
<td>143</td>
<td>28.1</td>
<td>56</td>
<td>400</td>
<td>ND</td>
<td>77</td>
<td>44</td>
</tr>
</tbody>
</table>

* Differential count of 300 nucleated cells.
† Findings during second blast crisis.
ND, bone marrow aspiration "dry tap." biopsy not made.

Cytogenetic studies of the bone marrow cells were performed in all patients, and in most cases, both "direct" and "culture" methods were used, as has been previously described. The constitutional karyotypes of the patients were determined from blood lymphocyte cultures stimulated by phytohemagglutinin (PHA) for 3–4 days.

RESULTS

In 9 of the 22 patients with adequate specimens available for membrane marker analysis, a significant proportion of the blasts (≥20%) expressed glycophorin-A (Table 2). In the remaining 13 patients, the blasts were glycophorin-A negative (≤5% staining with anti-GP-A).

The nine patients with glycophorin-A-positive blasts had the Philadelphia (Ph') chromosome. The peripheral blood and bone marrow findings during blast crisis are shown in Table 1. The age of the patients was 17–61 yr at the diagnosis of CML. The duration of the chronic or accelerated phase was 17–74 mo in 8 patients. After the onset of blast transformation, remission could not be induced in any of the 8 patients, and all died within 7 mo.

One patient (no. 4) presented with a Ph'-positive acute leukemia, which was initially interpreted as lymphoid blast crisis of CML. Immunofluorescent staining for glycophorin-A was not made at presentation. Remission period lasting 20 mo was achieved, whereafter a chronic phase of CML emerged in the peripheral blood. A second blast crisis with GP-A-positive cells developed 6 mo later.

The results of the surface marker analysis of the bone marrow or peripheral blood specimens are shown in Table 2. The surface membrane glycoprotein profile of the leukemic cells of two patients with a uniform glycophorin-A-positive blast population is shown in Fig. 1. In the immunoprecipitations with anti-glycophorin-A antiserum from the membrane lysates of the surface-labeled blasts, two bands corresponding to the dimeric and monomeric forms of glycophorin-A were precipitated (Fig. 1).

The surface expression of the DR antigen in the blasts was studied in six cases using indirect immunofluorescence or the Staphylococcus aureus rosetting method. The proportion of DR-positive blasts varied from 10% to 60%. In one case (patient no. 3), simultaneous expression of glycophorin-A and the DR antigen

Table 2. Phenotypic Cell Markers of the Glycophorin-A-Positive Blast Crisis

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Sample</th>
<th>Blasts (%)</th>
<th>Normoblasts (%)</th>
<th>Percent of All Cells</th>
<th>Percent of Blasts</th>
<th>HLA-DR</th>
<th>E-RFC†</th>
<th>sig</th>
<th>clg</th>
<th>EA-RFC†</th>
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<tbody>
<tr>
<td>(1)</td>
<td>PB*</td>
<td>82</td>
<td>0</td>
<td>80</td>
<td>100</td>
<td>—</td>
<td>2</td>
<td>20</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>(2)</td>
<td>BM</td>
<td>82</td>
<td>5</td>
<td>80</td>
<td>100</td>
<td>40†</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(3)</td>
<td>PB</td>
<td>86</td>
<td>0</td>
<td>90</td>
<td>100</td>
<td>60</td>
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<td></td>
</tr>
<tr>
<td>(4)</td>
<td>PB</td>
<td>19</td>
<td>3</td>
<td>20</td>
<td>100</td>
<td>10†</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(5)</td>
<td>BM</td>
<td>79</td>
<td>1</td>
<td>30</td>
<td>40</td>
<td>30</td>
<td>0</td>
<td>0</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>(6)</td>
<td>PB</td>
<td>98</td>
<td>0</td>
<td>30</td>
<td>30</td>
<td>—</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>(7)</td>
<td>BM</td>
<td>58</td>
<td>0</td>
<td>—</td>
<td>20†</td>
<td>20†</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>0</td>
</tr>
<tr>
<td>(8)</td>
<td>BM</td>
<td>75</td>
<td>6</td>
<td>30</td>
<td>40</td>
<td>30†</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
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<tr>
<td>(9)</td>
<td>PB</td>
<td>75</td>
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<td>30</td>
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<td>—</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

* PB, peripheral blood; BM, bone marrow.
† Assayed by the Staphylococcus aureus Cowan I rosetting technique, percent of the blasts positive.
‡ Percent of the blasts positive as assayed by the indicator red cells.
Fig. 1. Polyacrylamide slab gel electrophoresis patterns of surface-labeled leukemia cells and immune precipitates obtained with anti-glycophorin-A antiserum and control serum. (A) Standard proteins: M, myosin; PH, phosphorylase b; BSA, bovine serum albumin; OA, ovalbumin; CA, carbonic anhydrase. (B) Pattern of surface-labeled cells from patient no. 3, GP1O5, glycoprotein with an apparent mol wt of 10,500, etc. (C) Same as in B but exposed for a longer time to visualize minor bands. (D) Immune precipitate obtained with anti-glycophorin-A antiserum from cells shown in B and C; GPA-D, glycophorin-A dimer; GPA-M, glycophorin-A monomer. (E) Control obtained with nonimmune serum from an identical sample as in D. (F) Pattern of surface-labeled cells from patient no. 2. (G) Immune precipitate obtained with anti-glycophorin-A antiserum from cells shown in F. (H) Control with nonimmune serum from an identical sample as in H. The acrylamide concentration was 8%.

was seen in 60% of the blasts in double immunofluorescence using monoclonal anti-DR antibody and rabbit anti-glycophorin antiserum. The monoclonal anti-DR antibody precipitated two bands of molecules, with 29,000 and 34,000 apparent molecular weights, from the lysates of the surface-labeled blasts.

When the majority of the blasts expressed glycophorin-A, the blasts were morphologically uniform with several features reminiscent of erythroid precursors (Fig. 2A). The blasts were large or medium-sized cells, and they had a round or slightly indented, occasionally cleaved, nucleus usually with several nucleoli. The cytoplasm was deeply basophilic, and there was a distinct perinuclear halo. The plasma membrane was often folded, resulting in the formation of pseudopod-like projections. In alpha-naphthyl acetate esterase staining, the blasts showed a localized perinuclear positivity (Fig. 2B).

In one patient (no. 3), 30% of the uniformly glycophorin-A-positive basophilic blasts also stained with antiserum against coagulation factor-VIII-associated antigen, as an evidence for concomitant differentiation into the erythroid and megakaryocytic lineages (Fig. 2C).

In the bone marrow aspirates or biopsy specimens, normal erythroid maturation was decreased in most cases. In two patients (cases 2 and 3) with uniformly glycophorin-positive blasts, there were slight megaloblastoid changes.

The constitutional karyotypes of all nine patients with glycophorin-A-positive blasts were normal. In six patients, chromosomal changes consistent with blast transformation were found in bone marrow cells (Table 3). In two patients, two abnormal clones emerged in addition to the plain Ph' chromosome positive clone during blast crisis.

DISCUSSION

Chronic myelogenous leukemia is a clonal disorder involving the pluripotent hematopoietic stem cell. By karyotypic and glucose-6-phosphate dehydrogenase isoenzyme studies, it has been shown that the malignant clone includes granulocytic, monocytic, megakaryocytic, and erythroid lineages, as well as the lymphoid lineages.

According to conventional morphological and cytochemical criteria, the immature malignant cells accumulating during blast crisis are recognized as myeloid blasts in 50%–60% of cases and as lymphoid blasts in about 30% of cases. Using transmission electron microscopy-cytochemistry, megakaryoblastic and monoblastic blast transformations have also been identified. Electron microscopic examinations have revealed mixed blast populations showing phenotypical features of two or more lineages in up to 20% of blast crises of CML.

Erythroblastic transformation of CML diagnosed by the occurrence of blasts morphologically resembling megaloblastic proerythroblasts or basophilic erythroblasts as a majority of the malignant cells has been reported in 10% of blast crises. The classification of the malignant cells by morphology alone is not, how-
Fig. 2. (A) Morphology of the uniformly GP-A-positive blasts (from patient no. 3). (B) α-Naphthyl acetate esterase staining of GP-A-positive blasts (from patient no. 2). (C) Demonstration of intracytoplasmic factor-VIII-associated antigen by indirect immunofluorescence in uniformly GP-A-positive blasts (from patient no. 3).
Table 3. Cytogenetic Findings of the Patients With Erythroid Blast Crisis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Chronic Phase</th>
<th>Acceleration Phase or Blast Crisis</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>Not studied</td>
<td>46.XX,Ph(^1),t(9:22)</td>
</tr>
<tr>
<td>(2)</td>
<td>46,XY,Ph(^1),t(9:22)</td>
<td>46,XY,Ph(^1),t(9:22), -E, +i(Eq)</td>
</tr>
<tr>
<td>(3)</td>
<td>46,XX,Ph(^1),t(9:22)</td>
<td>46,XX,Ph(^1),t(9:22), -E, +i(Eq)</td>
</tr>
<tr>
<td>(4)</td>
<td>46,XX*</td>
<td>46,XX,Ph(^1),t(9:22)</td>
</tr>
<tr>
<td>(5)</td>
<td>46,XY,Ph(^1),t(9:22)</td>
<td>58,XY,Ph(^1),complex, + 22q-</td>
</tr>
<tr>
<td>(6)</td>
<td>46,XY,Ph(^1),t(9:22)</td>
<td>46,XY,Ph(^1),t(9:22), -17, +i(17q)</td>
</tr>
<tr>
<td>(7)</td>
<td>46,XX,Ph(^1),t(9:22)</td>
<td>46,XX,Ph(^1),t(9:22), -17, +i(17q), +8</td>
</tr>
<tr>
<td>(8)</td>
<td>46,XX,Ph(^1),t(3:9:22)</td>
<td>46,XX,Ph(^1),t(3:9:22)</td>
</tr>
<tr>
<td>(9)</td>
<td>46,XY,Ph(^1),t(9:22)</td>
<td>46,XY,Ph(^1),t(9:22), +X, +8, -17, +X, +8 +i(17q)</td>
</tr>
</tbody>
</table>

*At the primary acute phase, only Ph\(^1\)-positive cells and no normal mitoses were noted in the bone marrow.

ever, a reliable method to phenotype leukemias accurately.

By demonstrating the synthesis and surface expression of glycophorin-A in the leukemic cell line K562, derived from a patient with chronic myelogenous leukemia in blast crisis, Andersson et al. have shown that this cell line, which previously was considered to represent an undifferentiated blast, has a phenotype of an early erythroid precursor.\(^{19}\)

Our present findings indicate that erythroid blast transformation with a homogeneous blast population expressing glycophorin-A emerges rather frequently in CML, and mixed blast crises, with erythroid blasts as a minority, are also common. In contrast to our findings, Greaves and coworkers have reported glycophorin-A-positive blasts only in 2 of 33 cases of CML in blast crisis, using a monoclonal antibody against glycophorin-A.\(^{20}\)

Since carbohydrates constitute about 60% of the glycophorin-A molecule,\(^{21}\) sugar moieties presumably contribute to the epitopes recognized by some of the monoclonal antibodies. Our rabbit antiserum, on the other hand, also reacts with an incompletely glycosylated, precursor protein of glycophorin-A.\(^{23}\) It is possible that the rabbit anti-glycophorin antiserum reacts with some incomplete precursor molecules of glycophorin-A present on early stages of the erythroid maturation that cannot be detected with the presently available monoclonal reagents.

Previous studies on the occurrence of differentiation structures during normal erythropoiesis using monoclonal antibodies have revealed a coexpression of HLA-DR and GP-A in a very low proportion of erythroid precursor cells.\(^{3}\) We found significant numbers of malignant cells carrying both HLA-DR determinants and GP-A in two of six cases tested. Whether this represents "aberrant" expression due to the malignant transformation is unclear. Anyhow, the finding suggests a maturation arrest of the malignant blasts at an early stage of erythroid differentiation.

In one case there were findings suggestive of partial differentiation into the megakaryocytic lineage. Although the megakaryoblastic nature of the malignant cells was not confirmed by electron microscopy, a third of the blasts contained the coagulation factor-VIII-associated antigen, which is a marker for megakaryoblasts and more mature cells of the megakaryocytic lineage, intracytoplasmically.\(^{24,25}\) The simultaneous expression of the markers of both the erythroid and megakaryocytic lineages is in agreement with the findings from in vitro colony assays, where a common bipotential progenitor has been found.\(^{26,27}\)

The karyotypic findings of our patients are similar to those reported previously in the blast crisis of CML.\(^{28}\) No aberrations characteristic for the erythroid blast crisis were found. The close relationship between the emergence of the blast transformation and the appearance of the isochromosome 17q [or -E, +i(Eq)] reported previously\(^{29}\) was also seen in four of our patients.

The prognosis of the erythroblastic transformation of CML seems unfavorable. The failure to achieve remission and the short survival after the onset of the blast transformation, reported by Rosenthal et al.,\(^{18}\) was also seen in the majority of our patients.

REFERENCES

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Erythroid blast crisis in chronic myelogenous leukemia

M Ekblom, G Borgstrom, E von Willebrand, CG Gahmberg, P Vuopio and LC Andersson