CONCISE REPORT

Carbonic Anhydrase: A Marker for the Erythroid Phenotype in Acute Nonlymphocytic Leukemia

By Jami Walloch, Stanley Frankel, Mary Ann Hrisinko, and Susan C. Weil

Protein markers are often used to corroborate the morphological subtyping of hematopoietic malignancy. Most commonly, surface markers are used for the phenotyping of hematopoietic cells; however, internal proteins have also been used as markers. Glycophorin, hemoglobin A, hemoglobin F, and transferrin have all been used as markers for the erythroid phenotype. We have recently shown that carbonic anhydrase is constitutively and aberrantly expressed in two erythroleukemic cell lines. We here show that it is also present in high levels in primary erythroleukemic blasts and that it is a useful marker for the M6 phenotype when classifying acute nonlymphocytic leukemia.

IMMUNOHISTOCHEMISTRY is becoming increasingly more useful in the diagnostic categorization of acute leukemias. Most of the studies have been done on the acute lymphoblastic leukemias, in which a plethora of cell surface, cytoplasmic, and nuclear markers have been used for the characterization of lymphocyte type (B, T, or non-B, non-T cell) and degree of differentiation. The acute non-lymphocytic leukemias (M1 to M6, according to the French-American-British [FAB] classification) are less well immunohistochemically characterized; however, a significant body of data involving granulocytic and monocytic markers are accumulating. Much less phenotypic data have been gathered on the erythroleukemias (M6). This may be because so few leukemias are erythroleukemias: 95% of acute myeloid leukemias are M1 to M5; only 5% show early erythroid progenitors (M6).

Researchers have recently found erythroid markers useful in identifying early erythroid progenitors. The majority of the studies have been on hemoglobin (both adult and fetal) as a cytoplasmic marker. Transferrin has also been used as an erythroid marker, and Andersson et al and Greaves et al reported that a cell membrane glycoprotein, glycoprotein A, is specific for erythroid cells and that glycoprotein A appears early in primitive erythroid blasts.

Carbonic anhydrase (CA), the second most abundant protein in red blood cells, has been found at aberrantly high levels in early neoplastic erythroid blasts. While three isoenzymes of CA are found in mammalian erythrocytes, the work herein deals exclusively with the most abundant isoenzyme, CA1. In normal human and murine erythroid progenitor cells, a gradual and parallel increase in both CA and globin is observed during maturation. By contrast, in a murine erythroleukemic cell line (Friend or MEL cells) and a new human erythroleukemic cell line (HEL cells), CA is present at high levels in uninduced cells and does not change significantly with induction, whereas a marked increase in globin occurs with chemical induction. In this study, we ask whether this finding in two established continuous erythroleukemic cell lines can be extrapolated to primary human erythroleukemias and if CA may be an early cytoplasmic marker in human neoplastic erythroid cells. Herein, we report eight cases of erythroleukemia (FAB-M6) in which specimens were immunohistochemically stained for adult and fetal hemoglobin and CA.

MATERIALS AND METHODS

The B-5-fixed clot sections or bone core biopsies from eight patients with erythroleukemia (M6) diagnosed by classic morphological criteria were retrieved from cases diagnosed between 1977 and 1985. Also, the B-5-fixed clot sections from five cases each of M1 and M2 acute myelogenous leukemia were obtained. A modified avidin-biotinylated peroxidase complex immunohistochemical staining technique was used in an attempt to classify blast cells with respect to erythroid markers. Rabbit antiadult hemoglobin, antifetal hemoglobin, and anti-CA antibodies were used. The antihemoglobins (adult and fetal) were obtained from Dako (Santa Barbara, Calif); the anti-CA was produced in our laboratory as previously described. Briefly, New Zealand white rabbits were injected subcutaneously with 1 mg of CA-1 (Sigma Chemical Co, St Louis) emulsified in an equal volume of Freund’s adjuvant. Booster injections were given at 14-day intervals; rabbit serum was collected 14 days after booster injections and assayed by immunoblot analysis with the antigen. Nonimmunized rabbit serum was used as a negative control.

Specimens from each case were stained by immunoperoxidase with all three antibodies, and for each antibody, 500 erythroid cells were evaluated for the degree of positive staining (0 through ++ +). Each of these 500 cells was also evaluated for degree of maturity and classified as either early normoblasts or late normoblasts based on the degree of nuclear condensation.

RESULTS

The antihemoglobin antibodies and the anti-CA antibodies did not stain the blasts in the nonerythroid acute nonlymphocytic leukemias, M1 and M2. In these diseases, only rare residual late erythroid cells stained positively. In the erythroleukemias, many of the cells stained positively. For each antibody, 500 erythroid cells were evaluated and divided into early or late normoblasts. For each of the eight cases, a larger percentage of the erythroid cells stained positively with the anti-CA antibody than with either the antiadult or the antifetal hemoglobin antibody. A typical case is shown in Fig 1. The percentages (average and range) of positively stained cells (in each category) for all eight...
cases are shown in Table 1. The most striking result is that a significantly larger percentage of the early erythroblasts are strongly positive for CA than are positive for either of the hemoglobins (Table 1). This result indicates that the early neoplastic erythroblast has a high concentration of CA.

In the late erythroblasts, the number of cells positive for all three antibodies is similar, but the number more positive for CA is markedly higher than the number positive for the hemoglobin (Fig 1). These data suggest that although the production of hemoglobin increases during maturation of erythroblasts, the levels of CA in these neoplastic cells is initially high and remains high.

**DISCUSSION**

Although no previous work has examined primary erythroleukemic blasts with regard to CA expression, there is an extensive body of data on the CA expression in the peripheral blood cells of various hematologic malignancies. Several cases of juvenile chronic granulocytic leukemia have been described in which the mature red blood cells show a "reversion" to fetal erythropoiesis with high fetal hemoglobin levels and low CA levels. In addition, three cases (polycythemia vera, acute myelogenous leukemia, and erythroleukemia) have been described in which the red cells show high fetal hemoglobin but discordant levels of CA.

Our earlier studies showed that CA is abundantly expressed in the early neoplastic erythroblasts of the erythroleukemia HEL and MEL cell lines in vitro. This result led us to hypothesize that CA may be a more sensitive marker than hemoglobin for neoplastic erythroid cells in vivo. The results of this study (Table 1 and Fig 1) seem to corroborate our hypothesis. Anti-CA does not stain the nonerythroid acute nonlymphocytic leukemias (M1 and M2), but it does stain a significant number of M6 cells positively, especially in the early erythroblasts stage. Anti-CA seems to stain more cells positively in the early stage than does either adult or fetal antihemoglobin, especially if only the intensely staining cells are counted. And although the number of cells staining positively for all three antibodies is similar in the late erythroblast stage, if only the intensely staining cells are counted, anti-CA stains more cells positively than the anti-hemoglobin antibodies do.

Thus, the aberrant expression of CA has been observed both in vitro and in vivo in human erythroleukemias. The

### Table 1. Response of Erythroblasts From Erythroleukemic Patients to Staining With Antihemoglobins and Anti-CA

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>No. of Early Erythroblasts (Average)</th>
<th>No. of Late Erythroblasts (Average)</th>
<th>Positive Early Erythroblasts, + to +++ (%) Average (range)</th>
<th>Positive Late Erythroblasts, +++ Only (%) Average (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiadult hemoglobin</td>
<td>208 (128-320)</td>
<td>292 (180-366)</td>
<td>33.8 (10.2-70.4)</td>
<td>5.7 (0-27.8)</td>
</tr>
<tr>
<td>Antifetal hemoglobin</td>
<td>203 (125-277)</td>
<td>297 (223-375)</td>
<td>41.5 (5.2-73.7)</td>
<td>6.4 (0-28.6)</td>
</tr>
<tr>
<td>Anti-CA</td>
<td>210 (128-315)</td>
<td>290 (185-371)</td>
<td>65.1 (34.0-92.4)</td>
<td>30.6 (9.8-71.7)</td>
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appearance of abnormally high levels of CA in erythroleukemia cells may be explained by a change in gene regulation. Debure et al. described extensive sequence homology between the erb A gene of avian erythroblastosis virus and CA genes. It may be speculated that a mammalian oncogene exists that also has sequence homology with CA. An alternative hypothesis suggests that a translocation may juxtapose a CA gene in a manner analogous to the immunoglobulin-c-myC (q8.14) translocation in Burkitt’s lymphoma.

A case has been made for the usefulness of glycophorin A as a sensitive and specific marker for early erythroid blasts. Greaves et al. and Andersson et al. have found glycophorin A particularly useful in reclassifying "cryptic" erythroleukemias that were previously classified as de novo poorly differentiated acute myeloid leukemias (AML) or as acute lymphocytic leukemias (ALL). True AML and ALL seldom express glycophorin A. However, the use of glycophorin A requires fresh patient material, which may not be available for a case that has been misclassified. Thus, CA has the advantage as a marker for cryptic erythroleukemias because it can be used on fixed, paraffin-embedded materials.

Our data indicate that CA is a useful marker for erythroleukemia. For classification of acute nonlymphocytic leukemias, it is specific for M6 and more sensitive than either adult or fetal hemoglobin. An added advantage of CA as a marker is that it can be used on fixed, paraffin-embedded file material. CA may thus be useful in confirming erythroid phenotype and reclassifying poorly differentiated AML and ALL as M6.

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REFERENCES

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