Acute Nonlymphocytic Leukemia With Basophilic Differentiation

By Mark R. Wick, Chin-Yang Li, and Robert V. Pierre

Four cases of acute nonlymphocytic leukemia with primitive basophilic differentiation are presented. In all four cases, study revealed Philadelphia chromosome negativity, and in none were there clinical findings of chronic granulocytic leukemia. In each case, the leukemic blasts contained granules that failed to stain for peroxidase content but stained positively with toluidine blue. The former result could have led to the misclassification of the cases as lymphoid leukemias. Three of the four patients had physical findings that may have been due to circulating histamine excess. The histochemical and clinical features of these cases suggest that certain examples of leukemia with basophilic differentiation represent a distinctive variant of acute nonlymphocytic leukemia.

Although the term "basophilic leukemia" has been in diagnostic use for 75 yr,¹ the clinicopathologic features of this entity remain vaguely defined. Indeed, some authors have advocated abandoning such a nosologic category altogether, since most, if not all, cases previously designated as "basophilic leukemia" are now believed to represent a variant of chronic granulocytic leukemia.² ³

Recently, we saw four cases of acute nonlymphocytic leukemia in which primitive basophilic cellular differentiation was demonstrable; none of the patients had clinical or laboratory findings of antecedent or concomitant chronic granulocytic leukemia. Furthermore, physical and cytochemical findings in our four cases set them apart from other cases of acute nonlymphocytic leukemia. In this report, we enumerate these findings and discuss the relationship of previous reports of "basophilic leukemia" to the disease state observed in our patients.

MATERIALS AND METHODS

By prospective examination of Wright-stained bone marrow aspirates obtained at the Mayo Clinic from 1976 to 1981, all cases of acute leukemia seen at our institution during this 5 yr period were reviewed by two of us (C.Y.L. and R.V.P.). Light microscopic cytochemical stains, including those for peroxidase, Sudan black B, nonspecific esterase, and acid phosphatase reactivity, were used to further study each of these cases and to differentiate acute lymphocytic from acute nonlymphocytic leukemias.

In cases with leukemic granular blasts on Romanowsky stains, but with negative staining by the cytochemical methods listed above, toluidine blue stain was used (0.1% toluidine blue in 30% ethanol, with 3-min staining periods). In two such cases, staining with chloroacetate esterase and periodic acid Schiff (PAS) techniques was also done. Bone marrow aspirates from unrelated cases of known acute nonlymphocytic leukemia and acute lymphocytic leukemia were stained concurrently by the same methods and were used as controls. One case in which peroxidase-negative, toluidine-blue-positive granular blasts were observed was studied by electron microscopy. Aspirated bone marrow in this case was fixed in Trump's solution, buttoned, and processed routinely for ultrastructural analysis after staining with uranyl acetate and lead citrate. Ultrathin epoxy-embedded (Epon) sections also were stained with ruthenium red, using a previously described method.¹ Specimens stained conventionally and with ruthenium red were examined with a Philips 400 electron microscope.

RESULTS

Clinical Features

Using the above methods, four cases of acute nonlymphocytic leukemia with basophilic differentiation (morphological details given below) were identified (Table 1).

Pathologic Findings

Light Microscopy

The leukemic cells in each case were angular in contour with high nuclear-to-cytoplasmic ratios. The nuclei were acidophilic with finely dispersed chromatin and were oval or slightly lobulated. Each contained 1–3 eccentric, pale basophilic nucleoli. Cytoplasm was generally scant and was slightly basophilic or amphophilic (Fig. 1 A, C, E, and G). In the four cases, 10%–17% of the blasts contained azurophilic granules, which varied in size and number. Auer rods were not apparent. The marrow specimens contained 10%–20% normoblasts, some of which were dysplastic or multinucleated. The number of megakaryocytes was greatly decreased; mature eosinophils and basophils were absent. A few mature mast cells, enmeshed in reticulin-rich stroma, were evident in each case.

Cytochemical staining of the leukemic cells revealed uniform negativity with the peroxidase, Sudan black B, and α-naphthylbutyrate esterase methods (Table 2). In all four cases, the blasts demonstrated acid phosphatase positivity, which took on a diffuse staining pattern and was variable in strength. The cytoplasmic granules seen with Romanowsky stains also presented negative images with the peroxidase and Sudan black B techniques, as evidenced by counterstains used in

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Table 1. Clinical Features of Four Patients With Acute Nonlymphocytic Leukemia With Basophilic Differentiation

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex.</th>
<th>Age (yr)</th>
<th>Symptoms and Signs*</th>
<th>At Admission</th>
<th>Bone Marrow Findings†</th>
<th>Karyotype‡</th>
<th>Therapy§</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>72</td>
<td>Fever, weakness, 3 mo; bibasilar pneumonia; 2 wk</td>
<td>8.8 85</td>
<td>90% Blasts</td>
<td>47, XY (2 cells); 46, XY</td>
<td>i.v. VCN; p.o. 6-TG</td>
<td>Dead 47 days after diagnosis; no remission</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>43</td>
<td>Pruritic urticarial rash, focal cutaneous hyperpigmentation, 2 wk</td>
<td>10.0 3.3</td>
<td>90% Blasts</td>
<td>46, XX</td>
<td>i.v. ARA-C, DAUNO</td>
<td>Dead 50 days after diagnosis; no remission</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>57</td>
<td>Weakness, malaise, epistaxis, 3 wk; pruritic urticarial rash after induction persisting until death</td>
<td>9.5 3.2</td>
<td>80% Blasts</td>
<td>46, XY</td>
<td>i.v. ARA-C, DAUNO; i.v. VP 16-213 (second course)</td>
<td>Initial remission; relapse ending in death 6 mo after diagnosis</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>13</td>
<td>Fatigue, focal cutaneous hyperpigmentation, 6 mo</td>
<td>15.8 24.9</td>
<td>80% Blasts</td>
<td>46, XY</td>
<td>i.v. ARA-C, DAUNO; i.v. VCN, PRED, l-ASP, MTX (second course); i.v. VCN, MTX, ARA-C, 6-MP, PO PRED (maintenance)</td>
<td>Remission after second induction; maintained as of 1 yr after diagnosis</td>
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*Patients 1 through 3 free of lymphadenopathy and organomegaly; patient 4 had mild hepatosplenomegaly at diagnosis.
†Leukemic blasts in each case were sparsely granulated by Romanowsky staining; peroxidase, Sudan black B, and nonspecific esterase-negative; and positive with toluidine blue with metachromasia (see Table 2).
‡All cases lacked the Philadelphia chromosome.
§Therapy utilized because patient declined more aggressive treatment.

Profiles of rough endoplasmic reticulum were sparse and primitive in development. The presence of Golgi bodies was variable, but some cells possessed prominent structures of this type. Centrioles were likewise inconstantly seen.

The most notable features of these cells was their content of multivesicular bodies (Fig. 2), irregularly shaped large vesicles, and lysosomal granules of variable size and density. The latter organelles ranged from 150 to 900 nm in diameter; the smaller ones contained granular, uniform, electron-dense material, whereas larger lysosomes were more lucent, with a uniform, granulosphenical particulate matrix (Fig. 3). Some of them manifested a laminated, electron-dense substructure similar to myeloid bodies (Fig. 4), which was often admixed with a granular matrix within the same lysosome. Rarely, a bisecting line of membranous material divided the lysosomes into "theata"-like configurations. Intralysosomal crystals were not observed. Narrow skeins of fine parannuclear microtubules were apparent in many of the cells.

Electron Microscopy

Electron microscopy of leukemic cells in case 4 showed irregular cytoplasmic and nuclear outlines. Nuclear heterochromatin was moderately clumped and somewhat margined; most of the nuclei possessed at least one eccentric nucleolus, but well developed nucleolonemata were scarce. Occasional broad-based nuclear "blebs" could be seen.

The cytoplasm contained a modest complement of evenly dispersed mitochondria and polyribosomes. These organelles were further investigated with the toluidine blue stain. With the latter method, the granules were found to stain metachromatically (Fig. 1 B, D, F, and H). This reactivity varied in strength, according to nonuniformity in the number of granules in each case. However, cytochemical results of toluidine blue staining in unresected cases of acute lymphocytic leukemia showed that our study confirmed the presence of this marker. The latter organelles ranged in number from 1 to 900 nm in diameter; the smaller ones contained granular, uniform, electron-dense material, whereas larger lysosomes were more lucent, with a uniform, granulosphenical particulate matrix (Fig. 3).

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Ultrathin sections of blasts, in this case stained with ruthenium red, showed faint positivity within the matrix of lysosomes (Fig. 3, inset). Other organelles were nonreactive.
Fig. 1. (A, C, E, and G) Wright-stained bone marrow aspirates from cases 1 through 4, respectively. Coarse granules at present within cells (arrows) having blastic morphology. Granules vary in size and number throughout the four cases. A rough correlation was apparent between presence of presumed hyperhistaminemic symptoms and quantity of granulated blasts in any single patient. (Wright's stain; x400.) (B, D, F, and H) Toluidine-blue-stained bone marrow aspirates from cases 1 through 4, respectively. Granules seen with Wright's stain are observed to stain metachromatically (arrows). These same granules were negative with peroxidase, Sudan black B, and nonspecific esterase. Sum of histochemical results was consistent with basophilic differentiation for blastic cells (Toluidine blue; B, x160; D, F, and H, x 400.)
Table 2. Cytochemical Features in Four Cases of Acute Granulocytic Leukemia With Basophilic Differentiation*

<table>
<thead>
<tr>
<th>Case</th>
<th>Peroxidase</th>
<th>Sudan Black B</th>
<th>Acid Phosphatase</th>
<th>Nonspecific Esterase</th>
<th>Toluidine Blue</th>
<th>Periodic Acid Schiff</th>
<th>Chloroacetate Esterase</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>++: Diffuse</td>
<td>0</td>
<td>+</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>++: Diffuse</td>
<td>0</td>
<td>++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>+: Diffuse</td>
<td>0</td>
<td>+++</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>+++: Diffuse</td>
<td>0</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*0, less than 3% positive blasts; +, mild; ++, moderate; ++++, pronounced.

Fig. 2. Case 4. Electron micrograph of leukemic blast. Several free polyribosomes are present in cytoplasm, along with mitochondria, membrane-bound lysosomal granules (G), and a multivesicular body (MV). A broad-based nuclear bleb (B) is apparent at bottom of figure. (Uranyl acetate and lead citrate; x19,500.)
DISCUSSION

In 1906, Joachim\(^1\) described two patients with extreme basophilia and clinical features of myelocytic leukemia. This report has been followed by several others on “basophilic leukemia,” with or without the modifiers “acute,” “progranulocytic,” or “histioblastic.”\(^9,19\) Clinical findings and morphological features of many of these previous cases have been suboptimally delineated, precluding an assessment of whether they represented examples of acute nonlymphocytic leukemia, progranulocytic leukemia, chronic granulocytic leukemia,\(^9,19\) or acute lymphocytic leukemia.\(^29\) However, more current studies, notably that by Goh and Anderson,\(^7\) have suggested that virtually all cases of “basophilic leukemia” are a preterminal variant form of Ph\(^1\)-positive chronic granulocytic leukemia. A recent paper by Parkin and colleagues\(^21\) convincingly documented the features of six cases of blast crisis, in which the leukemic blasts had ultrastructural and ultracytochemical features of primitive basophils. However, all of these cases also showed Ph\(^1\) positivity and had clinical findings compatible with a diagnosis of previous or concomitant chronic granulocytic leukemia.

In contrast, our four cases appear to represent de novo cases of acute nonlymphocytic leukemia. In three, chromosomal findings were normal, and all four lacked
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Fig. 4. Case 4. Electron micrograph of leukemic blast, showing numerous lysosomal granules (G) containing both particulate matrices and membranous inclusions, the latter resembling myelinoid bodies (arrows). These features are consistent with basophilic lysosomal differentiation. (Uranyl acetate and lead citrate; x15,200.)

the Ph¹ chromosome. Although they may be examples of the Ph¹-negative form of chronic granulocytic leukemia, this is unlikely because in none of the cases were clinical histories or physical findings consistent with those of the latter disorder. The importance of these cases is threefold: first, cytochemical features of basophilic blasts may result in their misclassification as acute lymphocytic leukemia cells, with consequent errors in treatment; second, ultrastructural attributes of leukemic basophilic blasts are not totally analogous to those of mature basophils, as reported in previous cases of "basophilic leukemia"; and third, clinical findings in our four cases may provide a means for the separation of such cases from other examples of acute nonlymphocytic leukemia without basophilic differentiation.

Current recommendations for the cytochemical classification of leukemia have centered around myeloperoxidase activity in leukemic blasts as a criterion for differentiating acute lymphocytic leukemia from acute nonlymphocytic leukemia. Specifically, positivity for this staining technique in more than 3% of the blast population has been established as the diagnostic hallmark of acute nonlymphocytic leukemia. Alternatively, Sudan black B staining characteristics of leukemic myeloblasts are purported to reflect peroxidase
activity. Although they were of granulocytic origin, less than 3% of the leukemic cells in each of our four cases stained positively with either of these methods. A misdiagnosis of acute lymphocytic leukemia was avoided by attention to detectable granules in the cells on conventional Romanowsky staining. In that these granules stained metachromatically with the toluidine blue technique, a characteristic of basophilic granulocytes, the true nature of the leukemic blasts was ascertained. As noted above, Parkin et al. demonstrated light microscopic peroxidase negativity in several cases of "lymphoid" chronic granulocytic leukemia blast crisis, whereas ultrastructural study of these cases showed basophilic differentiation. Similar results were obtained by Marie and coworkers.

Several additional reports on the ultrastructural appearance and ultracltochemistry of basophilic granulocytes, both mature and immature, have appeared. In our case that was studied by these methods, sufficient electron microscopic similarities to well differentiated basophils were found to establish the lineage of the leukemic blasts. These included the uniform presence of cytoplasmic microtubules and multivesicular bodies, the variable development of Golgi bodies, and a characteristic appearance of lysosomal granules observed in the cells. The last-mentioned structures varied in size, and many contained a particulate matrix, sometimes mixed with membranous myelinoid figures. They were occasionally bisected by a fine membrane, yielding a "theta"-like configuration. However, the surface tubulovesicular structures, cytoplasmic glycosomes, and intralysosomal crystals seen in mature basophils were not evident in the leukemic cells.

By means of various ultracltochemical techniques, basophil granules have been shown to contain abundant sulfated mucopolysaccharide, little peroxidase, and copious acid phosphatase. The ruthenium red stain performed on electron microscopic sections in our case 4 is specific for mucopolysaccharide characteristically present in basophil granules and was positive in this case. Light microscopic stains confirmed the presence of acid phosphatase in the leukemic cells of all of our cases. Combined with their peroxidase negativity, the sum of these results is consistent with a basophilic differentiation for such cells.

We are aware that similar cytochemical results have been described in mast cell diseases and that some observers may choose to regard our cases as examples of mast cell precursor leukemia. Zucker-Franklin has provided ultrastructural data supporting a common histogenesis for basophils and mast cells and has observed much morphological overlap between immature cells of both lines. However, the light microscopic appearance of the leukemic cells in our four cases and the chloroacetate esterase negativity in two make a mast cell derivation unlikely.

Finally, in three of our four cases, there were clinical findings that were unlike those usually seen in other examples of acute nonlymphocytic leukemia. All three patients had cutaneous lesions, represented by a pruritic urticarial rash in two and patchy hyperpigmentation of the skin in two. One patient complained of severe headaches as well, similar to the "cluster" type. In each instance, these features were seen only during the active phase of disease. Denburg et al. have shown that immature basophils retain the capacity for histamine synthesis in cell culture. We postulate that the unusual physical features of our four patients were secondary to hyperhistaminemia. Although the synthetic abilities of the basophilic blasts were undoubtedly slight, the massive tumor load present in each case could have resulted in a cumulative histamine excess. We can only speculate as to the validity of these hypotheses, since histamine levels were not measured in any of our cases. However, the presence and severity of symptoms correlated with the number of toluidine-blue-positive granules observed among the four cases. In addition, similar signs and symptoms have been noted in patients with chronic granulocytic leukemia who have extreme basophilia. However, other hyperhistaminemic manifestations, such as bronchospasm, edema, dermatographism, diarrhea, and peptic ulceration, were not evident in our patients, as they have been in several of the aforementioned reports.

In summary, we have described four cases of acute nonlymphocytic leukemia with basophilic differentiation unassociated with chronic granulocytic leukemia. The leukemic blasts in each case were peroxidase-negative and Sudan-black-B-negative, but they contained granules on Romanowsky stains that had metachromatic tinctorial qualities using the toluidine blue stain. Ultrastructural and ultracltochemical studies, done on one of the four cases, supported a basophilic granulocyte origin for the leukemic cells. Three of the four patients had clinical manifestations of postulated hyperhistaminemia; two obtained remissions using a standard chemotherapy regimen for acute nonlymphocytic leukemia.

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