ULTRASTRUCTURAL PEROXIDASE CYTOCHEMISTRY OF LEUKEMIC CELLS

II. BLAST CRISIS OF CHRONIC MYELOGENOUS LEUKEMIA

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ABSTRACT

Eight cases of Philadelphia chromosome positive chronic myelogenous leukemia (CML) in blast crisis have been studied by ultrastructural peroxidase cytochemistry. In 7 cases, peroxidase reaction product was seen in the nuclear envelope and rough endoplasmic reticulum (RER) of the blasts. These cells had no cytoplasmic granules. In 6 cases, a few cells also showed peroxidase reaction product in the nuclear envelope, RER, and all cytoplasmic granules.

In contrast, peroxidase reaction product was not shown in the nuclear envelope and RER in all blasts in one case. The blasts did not have granules. Although these cells could not be identified, the high level of terminal deoxynucleotidyl transferase (TdT) activity suggested this case had undergone 'lymphoid' crisis.

Ultrastructural peroxidase cytochemistry is, therefore, useful for the identification of blast cells in CML-blast crisis.

INTRODUCTION

Despite effective treatments for the chronic phase of chronic myelogenous leukemia (CML), in the majority of the cases the disease undergoes the blast crisis which is characterized by an increasing proportion of blast cells in the bone marrow and peripheral blood.1

The origin of blast cells in CML-blast crisis sometimes remains controversial. Commonly a myeloblastic transformation occurs.1,2 Recently, a demonstration
of lack of enzymes of the myeloid cell line, a positive reaction with antiserum for common acute lymphocytic leukemia, and a high level of terminal deoxyribonucleotidyl transferase (TdT) activity have led to the possibility of a lymphoid form of blast crisis.\textsuperscript{3-5}

It is the purpose of this paper to identify the blast in CML-blast crisis by ultrastructural peroxidase cytochemistry.

MATERIALS AND METHODS

Patients: Eight patients with Philadelphia (Ph\textsuperscript{1}) chromosome positive CML in blastic transformation were examined. One patient (case 1) initially presented in blast crisis. Seven patients had the chronic phase, the duration of which ranged from 11 months to 147 months. Treatment for the chronic phase were busulfan, pipobroman, and dibromomannitol. No one underwent splenectomy. The criteria of blast crisis was the presence of 30 per cent or more blast forms and promyelocytes in the blood or 50 per cent or more blast forms and promyelocytes in the bone marrow aspirate.\textsuperscript{6,7}

Hematologic and cytogenetic findings and TdT activity are shown in Table 1. In two cases (case 1, 3), all blasts were peroxidase-negative by light microscopic cytochemistry. In the other cases, a few blasts were peroxidase-positive by light microscopic observation.

Methods: Ultrastructural peroxidase cytochemistry was previously described.\textsuperscript{8} Briefly, specimens obtained from peripheral blood and bone marrow were fixed with 1.5\% glutaraldehyde, then washed in 0.1 M Tris-HCl buffer, and pre-incubated in a solution of 0.05\% 3,3'-diaminobenzidine (DAB). After pre-incubation, incubation was performed in a medium containing DAB and H\textsubscript{2}O\textsubscript{2}. They were postfixed with OsO\textsubscript{4}, dehydrated, and embedded in Epon's mixture. Thin sections stained with lead citrate were prepared and examined under HU-12AS electron microscope.

RESULTS

Ultrastructural demonstration of peroxidase activity enabled several populations of blast in CML-blast crisis to be identified.

1 Myeloblasts. These cells showed peroxidase reaction product in the nuclear envelope and rough endoplasmic reticulum (RER) (Fig. 1). No cytoplasmic granule was found by observation of a series of thin sections. In general, they had a high nucleo-cytoplasmic ratio and round or oval nucleus with dispersed chromatin.
### Table 1

**Hematologic and cytogenetic findings and TdT activity in CML-blast crisis**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Peripheral blood</th>
<th>Bone marrow</th>
<th>Karyotype analysis</th>
<th>TdT activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hb (g/dl)</td>
<td>Platelet ($\times 10^4/\mu l$)</td>
<td>WBC (/\mu l)</td>
<td>Blasts (%)</td>
</tr>
<tr>
<td>1</td>
<td>41</td>
<td>M</td>
<td>7.7</td>
<td>3.2</td>
<td>50,100</td>
<td>73.0</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>M</td>
<td>6.9</td>
<td>3.0</td>
<td>30,500</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>58</td>
<td>M</td>
<td>9.2</td>
<td>4.7</td>
<td>8,700</td>
<td>73.0</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>M</td>
<td>4.7</td>
<td>2.5</td>
<td>277,000</td>
<td>91.5</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>F</td>
<td>8.5</td>
<td>154.0</td>
<td>13,400</td>
<td>53.0</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>M</td>
<td>14.4</td>
<td>7.5</td>
<td>92,800</td>
<td>35.0</td>
</tr>
<tr>
<td>7</td>
<td>35</td>
<td>M</td>
<td>7.8</td>
<td>3.5</td>
<td>11,600</td>
<td>27.0</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>F</td>
<td>7.6</td>
<td>27.3</td>
<td>7,400</td>
<td>37.0</td>
</tr>
</tbody>
</table>
These cells were observed in all cases except one case (case 1).
In case 3 whose blasts were peroxidase-negative by light microscopy, peroxidase reaction product was ultrastructurally detected in the nuclear envelope and RER of the blasts.

2 Promyelocytes. These cells were identified by the presence of myeloperoxidase activity in the nuclear envelope, RER and cytoplasmic granules (Fig. 2). They had a few granules in the cytoplasm. The number of granules per cell in a thin section was 3.6 ± 2.6 (mean ± S.D., range: 1–9). The smallest diameters of granules were measured from 150 to 400 nm in this study.

These cells were observed in 2–3% of the blasts in 6 cases (all cases except case 1, 3).

3 Basophil promyelocytes. These were recognized by either homogenous or stippled appearance of the peroxidase reaction in their granules (Fig. 3). Their granules were usually spherical; difficult to fix, and often extracted or disrupted by procedures.9

These cells were seen in about 5% of the blasts in 3 cases (case 2, 4, 8).

4 Undifferentiated blasts. In one case (case 1), all blasts had no granules in the cytoplasm and no peroxidase reaction product was demonstrated in the nuclear envelope and RER (Fig. 4).

DISCUSSION

Several descriptions can be found in the literature on the morphology of blast cells in the terminal phase of CML.7,10–14 Mathé et al.10 and Rosenthal et al.11 categorized morphologically as myeloblastic and lymphoblastic.

Conventional electron microscopic studies on the blasts of CML-blast crisis were carried out.7,12 Shaw et al.’s ultrastructural studies revealed a variety of findings, from very large, well-granulated cells, to completely agranular cells, with almost no cytoplasmic organelles.12

Peterson et al.7 reported that in 2 of the 28 cases they examined, ultrastructural features such as considerable nuclear lobulation and cytoplasmic granulation were indistinguishable from those seen in acute myelomonocytic leukemia; and in 2 cases the morphologic picture resembled acute lymphocytic leukemia.

Peroxidase which is present in azurophil granules of neutrophil polymorphs and monocytes, appears very early during maturation in the nuclear envelope, RER and Golgi apparatus before the development of granules in early precursors.14,15
Fig. 1  Myeloblast in CML-blast crisis. Peroxidase reaction product is seen in the nuclear envelope and rough endoplasmic reticulum (RER). $\times11000$

Fig. 2  Promyelocyte in CML-blast crisis. Peroxidase reaction product is detected in the nuclear envelope, RER and a few cytoplasmic granules. $\times7900$
Fig. 3 Basophil promyelocyte in CML-blast crisis. Peroxidase reaction product is seen in the nuclear envelope, RER and large stippled granules. Some granules are extracted or disrupted. ×11000

Fig. 4 Undifferentiated blast in CML-blast crisis. No peroxidase reaction product is demonstrated in the nuclear envelope and RER. No granule is seen in the cytoplasm. ×11000
Thus, non granulated myeloid precursors can be clearly distinguished from lymphoblasts.\textsuperscript{14,16} In a previous study, Palakavongs\textsuperscript{17} described myeloblasts in CML-blast crisis as being without cytochemically demonstrable myeloperoxidase activity. In this study, peroxidase reaction product was seen in the nuclear envelope and RER in nongranulated cells in CML-blast crisis. Hence, the nongranular cells which showed peroxidase reaction product in the nuclear envelope and RER can be identified as myeloblasts.

It is of interest that myeloperoxidase was detected ultrastructurally in the blasts which were totally peroxidase-negative by light microscopy in case 3.

It was reported in some cases that the blasts were monocytic rather than neutrophilic mainly on the basis of granule size.\textsuperscript{14,15} In the present study, the smallest diameter of granules in promyelocytes was 150 to 400 nm in size which, similar to that of AML,\textsuperscript{8} indicates that these cells were neutrophilic.

And the small number of granules in promyelocytes suggest an early granule formation.

In this study, basophil promyelocytes were also observed but did not occupy the predominant proportion as recently reported by Marie et al.\textsuperscript{14}

In case 1, all blasts demonstrated no reaction product for peroxidase. Although platelet peroxidase cytochemistry was not used in this study, these cells were not identified as megakaryoblasts because of the absence of abundant nuclear heterochromatin, α-granules and demarcation membranes.\textsuperscript{14,19}

A myeloperoxidase deficiency was noted in the terminal phase of CML,\textsuperscript{18,20} indicating that the possibility of myeloblasts lacking myeloperoxidase may be present in CML-blast crisis. They could be either common myeloid committed cells, myeloperoxidase deficient myeloblasts, or lymphoid precursors.

In case 1, a high level of TdT activity was suggestive of a lymphoblastic transformation. Recently, it has been reported that TdT activity is present in myeloblastic transformation of CML.\textsuperscript{31,22} It is of interest that the moderate level of TdT activity was found in myeloid crisis (case 6, 7).

Heterogeneity of morphological and cytochemical features in the blastic phase of CML support the hypothesis on the basis of immunological analysis\textsuperscript{23} that the target cell of CML-blast crisis is possibly a pluripotential stem cell or a closely related derivative.

Knowing the exact type of blast cells in CML-blast crisis should be important for the prognosis of the disease and for prospective and retrospective studies on its therapy.
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