Chronic Myelogenous Leukemia Cell Growth and Maturation in Liquid Culture

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SUMMARY

A recently developed liquid culture technique was used to determine the proliferative and maturational characteristics of leukemia cells from 7 patients with chronic myelogenous leukemia (CML). Marrow and blood leukocytes were cultured in an in vitro diffusion chamber for up to 45 days. Proliferation was assessed by viable cell counts and thymidine-3H-labeling indices. Maturation was determined using light and electron microscopy, histochemistry, phagocytosis, and tests for cell immunoglobulin receptors. CML cultures had viable cell counts far exceeding those of normal marrow cultures. The maturational pattern observed with CML in the chronic phase was qualitatively similar to that seen with normal bone marrow. Cultures consisted predominantly of mature granulocytes and macrophages. The macrophages were found to be functionally normal and the Philadelphia chromosome was identified in culture from two patients studied. We conclude that CML in the chronic phase is characterized in vitro by increased myelopoietic capacity with the potential for normal cellular maturation. These observations are consistent with the concept that increased cell production in CML results from an expanded committed stem cell pool. In blast transformation cellular differentiation is defective but, unlike acute myelogenous leukemia, cell proliferation remains high.

INTRODUCTION

CML is unique among the leukemias in that the disease has 2 distinct clinical phases and the Ph chromosome permits definitive identification of the leukemic cell line (4, 5, 22). Unlike AML, CML in the chronic phase is characterized by a greatly increased total granulocyte mass composed largely of mature elements (6, 9). The appearance and predominance of immature cells in association with progressive thrombocytopenia and anemia signals the onset of blast transformation which may appear similar to AML in clinical and morphological manifestations (2, 4).

The soft-gel technique for culturing bone marrow has provided an important means for studying human leukemic cells in vitro (15–20). Data obtained with this method indicate that some AML cells can replicate and differentiate in vitro and that they may respond to humoral stimulating factors (16, 17, 20). Chronic-phase CML cells will grow in semisolid culture (3, 16, 17) and form colonies containing cells with the Ph1 chromosome (7). Colony formation is dependent on a source of CSF.

We have used a recently developed liquid culture technique to examine the growth and maturational characteristics of hematopoietic cells from patients with CML. These in vitro studies indicate that CML in the chronic phase is characterized by increased myelopoietic capacity with normal potential for cellular maturation. In blast transformation proliferative activity remains high but cellular maturation is defective. These observations provide additional evidence that AML and the blast phase of CML are biologically distinct entities.

MATERIALS AND METHODS

Patients. Seven patients with Ph1-positive CML were studied (Table 1). Two patients had chronic-phase disease, 2 were in the accelerated or transitional stage (1 of whom had myelofibrosis) (14, 23, 25), and 3 were in the phase of blast transformation. All of the patients were anemic and none were on chemotherapy at the time of study. The patients with blast transformation all died within 4 weeks of the culture experiments. Data for normal bone marrow cultures were obtained from studies performed on 20 healthy volunteers.

Cell Culture. Bone marrow or peripheral blood leukocytes were isolated by sedimentation in dextran and centrifugation at 150 × g for 7 min. The cells were washed in complete culture medium and viable cell counts performed in trypan blue.

The in vitro diffusion chamber culture system has been described in detail previously (11). Briefly, the apparatus consisted of a small glass chamber closed at 1 end by dialysis membrane and mounted in an Erlenmeyer flask. Cultures were initiated with 3 × 10⁶ cells, and McCoy's 5A medium supplemented with 15% fetal calf serum and anti-
Table 1
Clinical characteristics of the patients studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Stage</th>
<th>Age</th>
<th>Sex</th>
<th>WBC count</th>
<th>Blasts (%)</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. T.</td>
<td>Chronic</td>
<td>42</td>
<td>F</td>
<td>355,000</td>
<td>3</td>
<td>900,000</td>
</tr>
<tr>
<td>J. P.</td>
<td>Chronic</td>
<td>32</td>
<td>F</td>
<td>8,000</td>
<td>3</td>
<td>470,000</td>
</tr>
<tr>
<td>V. C.</td>
<td>Transitional</td>
<td>66</td>
<td>M</td>
<td>156,000</td>
<td>32</td>
<td>416,000</td>
</tr>
<tr>
<td>B. H.</td>
<td>Transitional (myelofibrosis)</td>
<td>48</td>
<td>F</td>
<td>37,000</td>
<td>44</td>
<td>55,000</td>
</tr>
<tr>
<td>J. B.</td>
<td>Blast crisis</td>
<td>40</td>
<td>M</td>
<td>14,000</td>
<td>90</td>
<td>25,000</td>
</tr>
<tr>
<td>J. M.</td>
<td>Blast crisis</td>
<td>46</td>
<td>M</td>
<td>37,000</td>
<td>70</td>
<td>6,000</td>
</tr>
<tr>
<td>F. M.</td>
<td>Blast crisis</td>
<td>25</td>
<td>F</td>
<td>8,300</td>
<td>30</td>
<td>47,000</td>
</tr>
</tbody>
</table>

Biotics was used throughout. The culture flasks were incubated at 37° in a humidified environment with 7.5% CO₂.

Morphological and Functional Studies. Cultures were terminated at intervals up to 21 days, although occasional cultures were maintained for 45 days. Cells growing on the membrane and in suspension were pooled, counted in trypan blue, and deposited on glass slides with a cytocentrifuge (Shandon Southern Instruments, Sewickley, Pa.). Differential cell counts were performed on Giemsa-stained preparations. Identification of the various cell types was also based on morphological examination using the following histochemical stains: periodic acid-Schiff, methyl green-pyronin, peroxidase, lipase [a-naphthyl butyrate substrate (1)], and benzidine hemoglobin stain.

Cells were prepared for electron microscopy after fixation in cold buffered 1.5% glutaraldehyde and examined with a Siemens 1A instrument. Functional studies performed on living cultured cells included phagocytosis with both bacteria and fungi and tests for surface receptors for immunoglobulin (8). Radioautography was performed by exposing the cells to tritiated thymidine (1 μCi/ml) for 1 hr prior to terminating the cultures (11). Labeling indices were expressed as a percentage of total nucleated cells labeled based on counts of at least 300 cells. Immature cells were defined as blasts, progranulocytes, and promonocytes based on morphological and functional assessment (12). Cytogenetic studies were performed on 11-day cultures from 2 of the patients (J. P. and B. H.) after exposure to colcemid.

RESULTS

Cell Growth. Total viable cell counts as a function of time in culture are shown in Chart 1. In all of the patients studied cell numbers in vitro exceeded the upper range of normal established for marrow cultures performed on 20 healthy volunteers. A net increase of nucleated cells in vitro was noted in 5 of the 7 patients studied. The 2 patients with the lowest peripheral white cell count also had the lowest cell numbers in vitro.

Tritiated thymidine-labeling indices determined in 4 experiments are reported in Table 2. The number of cells incorporating label generally fell within the normal range. The uptake of thymidine-³H was shown to be due to prereplicative DNA synthesis by exposing cells to thymi-
Table 3  
Differential cell counts in vitro on cultures from the 2 patients with CML in whom cytogenetic studies were performed and a normal human bone marrow culture

<table>
<thead>
<tr>
<th>Patient</th>
<th>Days in culture</th>
<th>Blast cells</th>
<th>Promyelocytes and promonoocytes</th>
<th>Myelocyte to polymorphonuclear leukocyte</th>
<th>Eosinophiles</th>
<th>Lymphocytes</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. P. (chronic phase)</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>47</td>
<td>12</td>
<td>3</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>37</td>
<td>19</td>
<td>2</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>25</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>B. H. (myelofibrosis)</td>
<td>7</td>
<td>15</td>
<td>7</td>
<td>17</td>
<td>7</td>
<td>3</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>6</td>
<td>3</td>
<td>34</td>
<td>7</td>
<td>4</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>8</td>
<td>8</td>
<td>64</td>
</tr>
<tr>
<td>Normal subject</td>
<td>6</td>
<td>2</td>
<td>6</td>
<td>13</td>
<td>1</td>
<td>18</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>13</td>
<td>16</td>
<td>54</td>
</tr>
</tbody>
</table>

Viable and functional granulocytes and macrophages were observed after as long as 45 days of in vitro culture (Fig. 2).

**Cellular Maturation.** The differential cell counts in culture from the 2 experiments in which cytogenetic studies were performed are given in Table 3. Cell populations in vitro were qualitatively similar in chronic-phase CML and in normals (11), although granulocytes and eosinophiles were somewhat more prominent (Table 3). Granulocytic cells in all stages of maturation were observed, and the entire spectrum of monocyte-macrophage differentiation was seen. Macrophages were normal morphologically, histochemically, and as viewed by electron microscopy. These cells had normal phagocytic capacity and possessed surface receptors for IgG (Fig. 3).

The maturational patterns observed in vitro are illustrated in Chart 2. In the 2 patients with chronic-phase CML, cellular maturation in vitro was normal. Culture from patients with transitional or accelerated disease manifested a high percentage of immature cells early in culture, but by 14 days near-normal cellular differentiation was present. Patients in blast crisis showed defective cellular maturation in vitro; however, in only 1 case (J. B.) was the defect as complete as observed in AML (12).

**Cytogenetics.** In the 2 patients studied, direct cytogenetic preparations revealed the Ph1 chromosome in all evaluable mitoses observed in culture at 11 days. The presence of the Ph1 chromosome in vitro suggests that the abnormal cytogenetic characteristic was also present in bone marrow-derived macrophages as these cells were prominent in culture and were observed to replicate actively.

**DISCUSSION**

The culture studies described in this report were designed to determine the growth and maturational characteristics of neoplastic hematopoietic cells in patients with CML in its various stages. In CML total viable cell counts in vitro were 2 to 3 times greater than those observed in normals. A net increase in nucleated cells occurred in 5 of 7 of the CML culture studies. This contrasts sharply with data obtained using the same in vitro system to study AML cells where cell counts in culture were generally within the normal range (12).

Cell cycle times in CML as determined by several investigators have generally been normal or longer than those observed for normal myeloblasts (6, 19, 25). DNA synthesis times, however, are comparable to S phase estimates for normal granulocytic precursors (25). Also, data are available that indicate that CML myelocytes do not release mature granulocytes to the circulation more rapidly than normal (6, 9, 10). These observations, coupled with the high growth capacity in vitro and increased cell production in vivo, suggest that the primary defect in chronic-phase CML is an expansion of committed stem cell pools (6).

In the chronic phase of CML, cellular maturation in vitro as measured by the disappearance of immature cells...
was found to be normal, suggesting that the underlying pathophysiological lesion in CML is in cell proliferation rather than in the capacity for cell differentiation. Granulocytes and macrophages in culture were morphologically and functionally indistinguishable from those observed in cultures of normal bone marrow. The presence of the Ph1 chromosome in culture indicates that the neoplastic cell line was actively replicating. The cytogenetic studies also suggest that bone marrow-derived macrophages may contain the Ph1 chromosome and confirm cytogenetic data obtained by cloning CML cells in semisolid medium (7).

In studies on patients with accelerated or transitional disease, the ability to generate large numbers of progeny in vitro remained high but cellular differentiation was delayed. Cultures of peripheral blood from the patient with myelofibrosis showed a high percentage of immature cells early in culture but normal maturation occurred by 12 days in vitro. This observation suggests that immature cells with near-normal maturational capacity may appear in the peripheral blood of patients with abnormalities of bone marrow architecture.

The patients in blast crisis all showed defective cellular maturation in vitro (25) but, unlike AML cells, they maintained a high growth capacity. In only 1 case was the maturation defect as severe as that seen in AML (12). These observations suggest that the blast transformation phase of CML differs pathophysiologically from AML in that both a maturational and proliferative abnormality exists in blast crisis, whereas AML primarily represents a disturbance of cell differentiation (12).

Chronic-phase CML cells both produce and respond to CSF (13, 16, 17). Evidence is also available from in vivo studies indicating that in early CML humoral granulopoietic regulation may be operative (18, 24). Blast-phase CML cells proliferate actively in liquid culture but do not produce CSF (13, 17). Also, these blast cells show defective colony formation in semisolid culture, suggesting that they do not respond normally to CSF (16). These observations, coupled with those relating to the rare erythroleukoblastic crisis of CML (21), imply that blast transformation eventuates in an excessive proliferation of a clone of autonomous and maturationally defective hematopoietic cells (4, 5, 21, 22, 25).

ACKNOWLEDGMENTS
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REFERENCES

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Fig. 1. Myelocyte in telophase. Fourteen-day culture of CML cells. Giemsa stain, original magnification × 1200.

Fig. 2. Mature macrophages and granulocytes in culture at 45 days. Peroxidase stain, original magnification × 400.

Fig. 3. Rosette formation with CML macrophage (10-day culture) and anti-D-coated erythrocytes demonstrating presence of cell surface receptors for IgG.
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