Divergent Patterns of Marrow Cell Suspension Culture Growth in the Myeloid Leukemias: Correlation of In Vitro Findings With Clinical Features

By Laurence Elias and Peter Greenberg

Cellular recovery, maturation, and colony-forming cell (CFC) generation patterns of bone marrow cells from 23 patients with acute, subacute, and chronic myeloid leukemia (AML, SML, and CML) were studied using liquid and agar culture techniques. Increased recovery of proliferative myeloid cells from liquid culture was noted in 6 of 8 AML patients at diagnosis or relapse and 5 of 7 untreated SML patients. Patients with either AML or SML with rapid clinical progression exhibited greater recovery of cells in vitro with less maturation than patients with more stable disease. Studies from 3 patients with CML showed normal to increased cellular recovery with normal maturation. Three of 4 studies of AML patients followed sequentially in apparent remission, but with impending relapse, exhibited increased numbers of myeloblasts and promyelocytes, whereas 28 of 32 studies performed during stable remission were normal. The normally observed increase in CFC during liquid culture was absent in most leukemic marrow samples studied (3 of 4 AML, 4 of 6 SML, and 2 of 3 CML). Persistent low recovery of CFC during AML remission was associated in 3 patients with short remission duration. These studies indicated the potential utility of these techniques for the clinical evaluation of patients with myeloid leukemia and for studying factors involved in the progression of these diseases.

Development of in vitro culture techniques permitting the clonal growth of human marrow granulocytic progenitor cells in semisolid medium has provided insights into alterations of granulopoiesis in the myeloid leukemias. Liquid suspension culture has proved to be a useful method for assessing further the proliferative potential of myeloid cells in these diseases. Liquid culture technique has overcome certain limitations of the semisolid growth methods, namely, the inconsistency with which colony formation occurs in acute myeloid leukemia and the difficulty of harvesting the cultured cells from semisolid medium for further study. Furthermore, the finding that normal human bone marrow colony-forming cells (CFC) increase during liquid suspension culture in the presence of medium containing the humoral stimulatory substance, colony-stimulating factor (CSF), provides another in vitro parameter applicable to the evaluation of deranged states of granulopoiesis.

We have utilized suspension culture techniques to study patterns of cellular...
proliferation, maturation and CFC generation in patients with acute, sub-
acute, and chronic myeloid leukemia (AML, SML, and CML). Sequential
studies have also been performed in patients during remission of AML to define
alterations of these in vitro growth parameters as this disorder progresses
toward relapse.

MATERIALS AND METHODS

In Vitro Studies

Marrow specimens were assayed for agar colony-forming capacity utilizing human leukocyte
feeder layers as a source of colony-stimulating activity (CSA) as previously described.2 Culture
dishes were examined after 7-10 days of incubation; aggregates of more than 50 cells were scored
as colonies and aggregates of fewer than 50 were considered clusters. Normal values for colony-
forming capacity in our laboratory are $29 \pm 7.5 \times 10^3$ nucleated marrow cells (mean \( \pm \) SD).

Liquid suspension culture of marrow cells was performed by modifying previously described
techniques.7 Aliquots of $1 \times 10^6$ nucleated cells were placed in 10 x 35 mm plastic tissue
culture dishes (Falcon Plastics) in 2 ml of modified McCoy's 5-A medium containing 15\% fetal
calf serum with or without added 20\% human leukocyte conditioned medium as a source of CSA.
Replicate cultures were then incubated in a humidified air-CO\(_2\) environment.

Cells were harvested at periods of 1-8 days of culture by pipetting and were washed with
medium, centrifuged, and resuspended in culture medium. The harvested cells were counted in 2\% acetic
acid using a hemocytometer and their morphology and agar colony-forming capacity (triplicate
plating) were determined. Cell viability in individual experiments was checked by trypan
blue exclusion and found to be consistently greater than 90\%, during the period of culture. Differential
counts were performed on Wright-Giemsa stained slides prepared from cell suspensions
using a Shandon Elliot cytocentrifuge. All recoveries cited in the text and figures are absolute
numbers of cells or CFC present, normalized to the number initially placed into liquid culture. Thus percentage recoveries greater than 100\% represent absolute increases in numbers of cells or CFC.

Conditioned medium was prepared by incubating normal human peripheral blood mononuclear
cells, obtained by Ficoll Hypaque density centrifugation,11 at a concentration of $1-2 \times 10^6$
cells/ml in complete medium12,13 with added 2-mercaptoethanol (0.5 mM). After cellular incuba-
tion for 7 days under standard conditions, the supernatant was harvested, filtered through a
0.45-\(\mu\)m Millipore filter, and stored at -20\(^\circ\)C until use. Batches of conditioned medium were
assayed for their ability to stimulate human marrow colony formation in monolayer agar culture
prior to their use in liquid culture. The batches of conditioned medium employed in these ex-
periments had comparable CSA.

For statistical analyses, \( p \) values refer to two-tailed Student's \( t \) test, except where noted.

Patient Population

Control marrow samples were obtained from eight subjects with normal granulopoiesis (six pa-
tients undergoing general anesthesia for cardiovascular surgery, one patient with iron deficiency
anemia, and one patient with benign lymphadenopathy). Due to technical mishaps, cell recovery
data were available from seven studies, and CFC recovery data from six studies. The patients
studied included eight with AML at diagnosis or relapse, nine with AML in remission (four
studied in relapse as well), seven with SML, and three with CML.

Criteria for determining clinical status in AML have been described previously.14 The terms M-1,
M-2, and M-3 marrows were defined, respectively, as less than 5\%, 5\% - 25\%, and greater than 25\%
myeloblasts in marrow aspirates. The patients with AML were treated with a chemotherapeutic
induction regimen which included daunorubicin, cytosine arabinoside, and 6-thioguanine.15 Seven
of the patients with AML in remission received monthly maintenance chemotherapy consisting of
2-day courses of cytosine arabinoside and 6-thioguanine,15 while two patients received no chemo-
therapy during remission. Bone marrow aspirates were obtained for clinical evaluation and marrow
cultures at monthly intervals during remission just prior to chemotherapy.

The clinical criteria characterizing patients with SML have been described previously.16-20
These patients exhibited an increase in the percentage and number of marrow myeloblasts (10^6 to 40^6, with greater myeloid maturation than that present in AML, and they had relatively indolent courses. Single or multiple refractory cytopenias and qualitative abnormalities of the erythroid, granulocytic, and/or megakaryocytic lines were also frequently present. Table 4 shows the clinical features of this group of patients. Marrows all showed normal or increased cellularity. Leukocyte alkaline phosphatase was decreased in four of the five cases in which it was determined, and serum muramidase was increased in four of four cases. Marrows from patients with SML and CML were obtained prior to the institution of chemotherapy. All bone marrow samples were obtained after receiving informed consent in accordance with guidelines set by the Stanford Medical Center Human Experimentation Committee.

RESULTS

Cellular Recovery and Maturation in Suspension Culture

Control subjects. Figure 1 indicates the recovery of total marrow cells, proliferative myeloid cells (myeloblasts, promyelocytes, and myelocytes), and nonproliferative myeloid cells (metamyelocytes, bands, and polymorphonuclear leukocytes) on successive days of liquid culture from seven of the eight control subjects. This proliferative pattern was unaffected by the presence of conditioned medium in culture (Table 1). Monocytes and macrophages were present in small numbers (< 15%) during the period of culture, the remainder essentially being maturing myeloid cells. Values (mean ± SD) for peak recovery during the culture period were as follows: total cells, 44 ± 11%; proliferative myeloids, 53 ± 14%; and nonproliferative myeloids, 40 ± 18%. Thus, except for a slight enrichment of proliferative myeloids, the proportion of myeloid cells of each type during the 7-day culture period was similar to that of normal marrow.

AML diagnosis/relapse. Figure 2 depicts the total cell, proliferative, and nonproliferative myeloid cell recovery curves of studies from nine bone marrow samples from patients with AML at diagnosis (three patients) or relapse (five patients, six determinations). Accumulation of increased numbers of immature myeloid cells during culture was noted in six of the eight patients. The two patients (indicated as ○ in figures) who exhibited less in vitro growth of immature cells with better maturation had slowly progressive disease. Increases in numbers of cells in liquid culture of marrow from AML patients occurred only in cultures containing conditioned medium (Table 2, Fig. 3).

In six studies, recovery of nonproliferative myeloid cells was within the normal range. Nonproliferative myeloid cells, however, were present in small numbers, and maturation was considered defective in view of the large and increasing numbers of immature myeloid precursors. In two cases nonproliferative myeloid cells were absent during culture.

AML in remission. Thirty-five determinations of cellular recovery and maturation in liquid culture were performed serially from marrow cells of nine patients with AML in remission. Thirty-two studies performed more than 6 wk prior to relapse were characterized by normal (28 studies) or slightly abnormal (4 studies) patterns of proliferation and maturation. Three of four studies of marrow from three patients obtained within 6 wk of clinical relapse exhibited excess accumulation of immature cells (p < 0.025, χ² corrected). (See Fig. 4A-1 and B-1, Table 3).
*Mean ± SD of recovery at day 5 of culture. —CM, cultured without conditioned medium; +CM, cultured with 20% conditioned medium.

Table 1. Effect of Leukocyte Conditioned Medium on Growth Patterns of Normal Marrow in Suspension Culture

<table>
<thead>
<tr>
<th></th>
<th>Cellular Recovery After Liquid Culture*</th>
<th>Number of Studies</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cell recovery (%)</td>
<td>—CM  29.3 ± 10.3</td>
<td>7</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>+CM  35.9 ± 22.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CFC recovery (%)</td>
<td>—CM  99.5 ± 45.7</td>
<td>6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>+CM  188.2 ± 35.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Growth patterns in liquid culture of normal control marrows. (A) Cell recovery. (B) Proliferative myeloid cell recovery. (C) Nonproliferative myeloid cell recovery. (D) CFC recovery. Points represent mean ± SD of results from eight experiments in the presence of leukocyte conditioned medium. Recoveries are expressed as percentages of absolute numbers of cells present at initiation of cultures (day 0 = 100%; any recovery >100% represents absolute increase in cells or CFC).
Fig. 2. Growth patterns in liquid culture of AML marrow cells at diagnosis or relapse. (A) Cell recovery. (B) Proliferative myeloid cell recovery. (C) Nonproliferative myeloid cell recovery. (D) CFC recovery. Circled points indicate studies of patients 7 and 8 (Table 2). Shaded areas represent normal values (mean ± SD). Recoveries are expressed as percentages of absolute numbers of cells present at initiation of cultures (day 0 = 100%; any recovery >100% represents absolute increase in cells or CFC).

Table 2. Patients With Acute Myeloid Leukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Marrow</th>
<th>Status at Time</th>
<th>CFC/10⁵ Nucleated Cells</th>
<th>Peak Cell Recovery (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Morphology</td>
<td>Study Obtained</td>
<td></td>
<td>− CM</td>
</tr>
<tr>
<td>1 (a)</td>
<td>M-2</td>
<td>First relapse</td>
<td>49</td>
<td>132</td>
</tr>
<tr>
<td>2</td>
<td>M-2</td>
<td>First relapse</td>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>M-2</td>
<td>Second relapse</td>
<td>63</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>M-3</td>
<td>Second relapse</td>
<td>58</td>
<td>141</td>
</tr>
<tr>
<td>5</td>
<td>M-3</td>
<td>Diagnosis</td>
<td>1</td>
<td>67</td>
</tr>
<tr>
<td>6</td>
<td>M-3</td>
<td>Diagnosis</td>
<td>0 (&gt;400 clusters)</td>
<td>117</td>
</tr>
<tr>
<td>7</td>
<td>M-3</td>
<td>Diagnosis</td>
<td>4</td>
<td>73</td>
</tr>
<tr>
<td>8</td>
<td>M-3</td>
<td>First relapse</td>
<td>42</td>
<td>46</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td>74 ± 29</td>
</tr>
</tbody>
</table>

*p < 0.05

* − CM, cultured without conditioned medium; + CM, cultured with 20% conditioned medium.
Subacute myeloid leukemia (SML). The recoveries of total cells and proliferative and nonproliferative myeloid cells in the seven patients with SML are presented in Fig. 5. Three patients (patients 5–7) in a progressive phase of their disease (increasing leukocytosis, splenomegaly, and systemic symptoms) exhibited enhanced recovery of immature cells and markedly defective maturation. In contrast, two of the four patients with clinically stable disease exhibited lower rates of increase of immature cells and all four had greater evidence of maturation. See also Table 4.

Chronic myeloid leukemia (CML). Three patients with CML were studied. Marrow cells obtained from these three patients had increased myeloid cell proliferation (peak cell recovery of 52%, 89%, and 146%, respectively) associated with normal maturation.

CFC Recovery After Liquid Culture

Normals. The normal range for CFC recovery was determined from studies of marrow cells from six control subjects (Fig. 1D). The peak recovery of CFC was 207% ± 57% (mean ± SD) noted between days 5 and 7 of culture. The increased recovery of CFC occurred in the presence of conditioned medium in the culture (Table 1). CFC recovery was not related to the initial colony-forming capacity in the control or pathologic marrow samples studied.

AML diagnosis/relapse. CFC generation studies were performed in four
Fig. 4. Serial studies of nine patients with AML during remission. (A) Patients having remissions longer than 4 mo in duration. (B) Patients having remission of 4 mo or less. (1) Peak recovery of blasts and promyelocytes from liquid culture. (2) Peak recovery of CFC from liquid culture. (3) CFC/10^5 nucleated marrow cells. Shaded areas represent normal values (mean ± 25D). M-1 marrows; M-2 marrows; M-3 marrows.

Table 3. Acute Myeloid Leukemia Proliferation of Blasts and Promyelocytes in Liquid Culture

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Remission Stable</th>
<th>&lt; 6 wk Prior to Relapse</th>
<th>Relapse</th>
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<tr>
<td>Peak recovery (%)*</td>
<td>69 ± 70</td>
<td>110 ± 76</td>
<td>418 ± 153</td>
<td>249 ± 136</td>
</tr>
<tr>
<td>Number of studies</td>
<td>7</td>
<td>32</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>p values, as compared to</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal subjects</td>
<td>—</td>
<td>NS</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Stable remission</td>
<td>NS</td>
<td>—</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Impending relapse</td>
<td>&lt; 0.01</td>
<td>&lt; 0.001</td>
<td>—</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Mean ± SD, determined after 5-7 days of culture.
### Table 4. Patients With Subacute Myeloid Leukemia

<table>
<thead>
<tr>
<th>Patient</th>
<th>Leukocytes</th>
<th>Neutrophils</th>
<th>Monocytes</th>
<th>Blasts</th>
<th>Platelets</th>
<th>Hct (%)</th>
<th>LAP</th>
<th>Muramidase</th>
<th>Splenomegaly</th>
<th>Cellularity</th>
<th>Myeloid</th>
<th>Erythroid</th>
<th>Megakaryocytes</th>
<th>CFC/10⁵ Nucleated Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.0</td>
<td>1.0</td>
<td>0.0</td>
<td>0.0</td>
<td>320</td>
<td>20</td>
<td>N</td>
<td>ND</td>
<td>No</td>
<td>I</td>
<td>I</td>
<td>D</td>
<td>I</td>
<td>0; (230)*</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>1.9</td>
<td>1.2</td>
<td>0.0</td>
<td>18</td>
<td>33</td>
<td>D</td>
<td>I</td>
<td>Yes</td>
<td>N</td>
<td>I</td>
<td>D</td>
<td>I</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>1.8</td>
<td>0.4</td>
<td>0.0</td>
<td>72</td>
<td>35</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>I</td>
<td>I</td>
<td>D</td>
<td>N</td>
<td>0; (21)*</td>
</tr>
<tr>
<td>4</td>
<td>1.9</td>
<td>0.7</td>
<td>0.3</td>
<td>0.0</td>
<td>19</td>
<td>30</td>
<td>ND</td>
<td>ND</td>
<td>No</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>34.5</td>
<td>15.5</td>
<td>3.5</td>
<td>1.0</td>
<td>97</td>
<td>22</td>
<td>D</td>
<td>I</td>
<td>Yes</td>
<td>I</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>0; (70)*</td>
</tr>
<tr>
<td>6</td>
<td>150.0</td>
<td>67.5</td>
<td>24.0</td>
<td>25.0</td>
<td>42</td>
<td>23</td>
<td>D</td>
<td>I</td>
<td>Yes</td>
<td>I</td>
<td>I</td>
<td>D</td>
<td>D</td>
<td>0; (&gt; 400)*</td>
</tr>
<tr>
<td>7</td>
<td>9.6</td>
<td>3.5</td>
<td>1.9</td>
<td>0.2</td>
<td>17</td>
<td>22</td>
<td>D</td>
<td>I</td>
<td>Yes</td>
<td>I</td>
<td>I</td>
<td>D</td>
<td>N</td>
<td>140</td>
</tr>
</tbody>
</table>

Patients 1–4 were hematologically stable, while patients 5–7 had enlarging spleens, increasing leukocytosis, and systemic symptoms at the time of study. The peripheral blood of patients 5 and 6 included a significant number of intermediate myeloid cells as well as the cell types enumerated above.

Abbreviations: LAP, leukocyte alkaline phosphatase; N, normal; I, increased; D, decreased; ND, not done.

*Clusters.
Fig. 5. Growth patterns in liquid culture of SML marrow cells. (A) Cell recovery. (B) Proliferative myeloid cell recovery. (C) Nonproliferative myeloid cell recovery. (D) CFC recovery. Shaded areas represent normal values (mean ± SD). Recoveries are expressed as percentages of absolute numbers of cells present at initiation of cultures (day 0 = 100%; any recovery >100% represents absolute increase in cells or CFC). Numbers refer to patients identified in Table 4.

patients with enumerable agar colony formation prior to culture (Fig. 2D). Three of the four studies showed low CFC recovery after liquid culture. The patient (indicated by ◊) whose CFC recovery was high also lacked increased immature myeloid cell proliferation and had a prolonged partial relapse prior to the time of study.

AML remission. As shown in Figure 4A-2 and B-2, the three patients followed sequentially who had relatively short remissions (≤4 mo) had consistently low values of peak CFC recovery (92° ± 18°, mean ± SD) which were significantly lower than those from the six patients having longer remissions (153° ± 54°, p < 0.01). Similarly, 6 of 10 studies from patients with short remissions showed low CFC generation as compared to only 5 of 34 in the patients with long remissions (p < 0.01, χ² corrected). The low values observed
in patients with short remissions were unrelated to their neutrophil counts. Low values observed in the patients with long remissions occurred in patients whose marrows remained moderately hypocellular or who were neutropenic at the time of study. Among the patients with long remissions and normocellular bone marrows, peak CFC recovery was lower in neutropenic patients than in non-neutropenic patients (139\(\pm\) 39\(\%\) versus 185\(\pm\) 49\(\%\); \(p < 0.01\)). The latter value for studies of the non-neutropenic patients was not significantly different from normal. There was no significant difference in mean values of peak CFC recovery from the four patients with long remissions receiving chemotherapy compared to the two patients not receiving maintenance chemotherapy.

**Subacute myeloid leukemia (SML).** Marrow cells which produced colonies in agar prior to liquid culture continued to do so after liquid culture, and cluster-forming marrow cells persisted in producing clusters. As shown in Fig. 5D, the three SML marrows with initial colony formation (patients, 2, 4, and 7) exhibited normal or slightly decreased CFC recovery. Recovery of cluster-forming cells was abnormal in three patients with initial cluster formation.

**Chronic myeloid leukemia (CML).** Peak CFC recovery from liquid culture was high in one patient (330\(\%\)) and low in two patients (27\(\%\) and 42\(\%\), respectively) with CML.

**DISCUSSION**

Our studies delineated growth patterns in liquid culture of morphologically recognizable myeloid cells and their precursors in normal subjects and in patients with acute, subacute, and chronic myeloid leukemia. Marrow from normal subjects showed decreasing myeloid cells and increasing CFC during suspension culture, confirming and extending previously described studies by other workers.\(^7\) Recovery of normal marrow CFC was again shown to be greater in the presence of exogenously added conditioned medium than in its absence.\(^7\) The lack of a wave of increased maturing myeloid cells following the CFC peak in the conditioned medium containing cultures may have been due to the relatively short period of incubation.\(^6\)

Immature myeloid cells from six of eight patients with AML at the time of diagnosis or relapse exhibited exponential proliferation in the presence of conditioned medium, with deficient maturation. Two patients who had slowly progressive disease exhibited low recoveries of immature cells associated with greater maturation. The increased cellular recovery (compared to normals) of most leukemic marrows may have been due to the presence of a higher proportion of immature cells with greater proliferative capability in the leukemic marrows or to differences in their responsiveness to factors in conditioned medium.\(^2\)

The recovery of CFC from suspension culture was low in three AML patients early in relapse (Fig. 2). Since these patients also exhibited markedly decreased colony-forming capacity prior to liquid culture when they progressed to a more advanced stage of relapse, it was likely that the CFC observed in these cases arose from residual normal clones. If so, the low CFC recovery observed could represent an inhibitory effect on normal progenitor cells by the leukemic cells.\(^2,23,24\) Alternatively, the CFC observed during relapse may have derived
from leukemic cells with a greater potential for differentiation but abnormal proliferation in liquid culture.

Patients evaluated serially during remission of AML had normal patterns of myeloid cell maturation and cellular recovery until 2-6 wk prior to relapse, at which time impaired myeloid maturation became evident. Since the patterns in vitro at the time of impending relapse were similar to those noted during relapse, this finding may reflect proliferation of emerging leukemic cells. During remission, persistent low CFC recovery from liquid culture was associated with relapse within a relatively short period of time (4 mo) in three patients, whereas patients having longer remissions had normal CFC recovery. Such measurements appear useful for assessing the adequacy of clinical remission in AML.

Patients with SML having clinical evidence of progression exhibited increased recovery of immature cells in suspension culture associated with decreased myeloid maturation. Patients in a more stable phase of their disease exhibited less growth of immature cells with greater maturation. As was the case with AML, marrow from patients with SML generally had abnormal CFC or cluster-forming cell recovery after suspension culture.

Considerable overlap existed in growth patterns of AML and SML marrow cells in liquid culture. Patients with classical AML and progressing SML exhibited increased proliferative myeloid cell recovery associated with decreased maturation in liquid culture. Conversely, the patients with AML with slowly progressive disease and the stable SML patients had lower in vitro cellular recovery with relatively greater maturation. The observed overlap in growth patterns in AML and SML reflected a degree of in vitro cellular kinetic variation among patients which correlated with their clinical courses, seemingly independent of differences in myeloid maturation in vivo.

Marrow cells from three patients with CML exhibited normal myeloid maturation patterns and increased or decreased recovery of CFC. These data confirmed recent studies by others.

These results indicate that the suspension culture technique has value in the clinical evaluation of patients with myeloid leukemias and in the study of factors involved in the progression of these diseases, as variations in rate of disease progression are reflected by differences in patterns of growth in vitro. Furthermore, it will be of interest to elucidate in future investigations the mechanism involved in the above-described abnormalities in CFC recovery from liquid culture.

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REFERENCES

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