Diagnostic and Prognostic Significance of the CFU-c Assay in Acute Nonlymphoblastic Leukemia

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Abstract

A simplified system for classification of aggregate incidence and growth pattern in the CFU-c (colony-forming units in culture) assay, allowing simple and reproducible interpretation of test results, was developed and applied to 552 bone marrow samples from 202 adult patients with acute leukemia. Ninety-six consecutive patients with acute nonlymphoblastic leukemia were studied at diagnosis. The microcluster growth pattern ("acute myeloid leukemia-type") found in 57% of the patients, was significantly associated with higher remission induction rates on both protocols ($p = 0.004$). No relationship between growth pattern at diagnosis and remission duration was observed. The acute myeloid leukemia-type growth pattern was found to be more frequent in leukemias exhibiting morphological evidence for partial myeloid or monocytic differentiation. The favorable prognostic significance of Auer rods previously described was recognized in two CFU-c growth pattern categories. Of patients exhibiting an acute myeloid leukemia-growth pattern and Auer rods, 89% obtained complete remissions compared to 38% in the Auer rod-negative group showing other growth pattern variants. The CFU-c assays performed during complete remission on 354 samples of 48 patients with acute nonlymphoblastic leukemia and, as a control, on 85 samples of 43 patients with acute lymphoblastic leukemia revealed marked spontaneous as well as chemotherapy-related fluctuations of aggregate incidence and growth pattern. These and similar observations obtained with other assay systems are probably of major pathophysiological significance but preclude clinical application of the CFU-c assay to the monitoring of remission status in patients with acute nonlymphoblastic leukemia.

Introduction

In recent years, in vitro clonal assay systems for analysis of hematopoietic progenitor cells have been increasingly used in the diagnostic, prognostic, and pathophysiological analysis of human leukemias and related disorders (16, 22, 30). The CFU-c2 assay for committed myeloid progenitor cells has been most widely used in the clinical diagnostic and prognostic evaluation of patients with ANLL (20, 28, 30), MDS ("preleukemia") (14, 21), chronic myeloid leukemia (18, 27), polycythemia vera and myelofibrosis (19), as well as in patients undergoing bone marrow transplantation (25, 30). In addition, valuable information about regulatory interactions between hematopoietic progenitor cells and their progeny has been obtained using the CFU-c assay (4, 5, 9, 14, 23), often in conjunction with other phenotypic marker techniques (11, 12), advancing the understanding of pathogenesis and pathophysiology of human leukemias. Several classification systems have been proposed for ANLL, based on aggregate incidence and growth pattern of leukemic stem cells in the CFU-c assay, and have been shown to be of prognostic significance with respect to remission induction rates (20, 26, 29) and remission duration (8). This study was designed to assess the prognostic significance of the CFU-c assay in ANLL at presentation and during CR and its relationship to conventional cytological and cytochemical criteria. In addition, a simplified system for classification of CFU-c incidence and growth pattern was developed to evaluate the feasibility of using the standard CFU-c assay as a routine clinical diagnostic procedure of sufficient reproducibility to be performed by technical personnel without the constant need for highly skilled professionals for scoring and interpretation.

Materials and Methods

All patients studied were diagnosed and followed by the Hematology/Lymphoma Service, Memorial Hospital. Ninety-six patients with ANLL were studied at diagnosis. Of these, 48 patients subsequently received chemotherapy on the L-12 protocol (10), and the remaining 48 patients were on the L-14 protocol (2). Fifteen patients with ALL were studied at presentation and then treated with the L-10/L-10M protocol (28), as were 43 patients with ALL studied during CR. The 48 patients with ANLL investigated in CR were receiving maintenance chemotherapy on the L-6 ($n = 10$ (6)) and the L-12 ($n = 38$ (10)) protocols. Initial bone marrow smears of all patients were reviewed and classified according to the recently described modification of the FAB system (15). The CFU-c assay was performed on bone marrow aspirates as described previously (26). Cultures were scored without knowledge of diagnosis or clinical status after 7 days of incubation, and the incidence per $10^6$ cells of colonies (aggregates with $\geq 40$ cells) and clusters (aggregates with $< 40$ cells) was recorded. Aggregate incidence and growth pattern were then classified as "no growth" in the absence of significant aggregate formation and as "normal" when less than 50 colonies and less than 1000 clusters were counted with a less than 30-fold excess of clusters (Table 1). The most frequent growth pattern observed in ANLL was termed "leukemic" or "AML type" and revealed a low colony incidence and a more than 30-fold cluster excess. In three of the 96 ANLL cases studied, a very high colony and cluster incidence, but with a colony to cluster ratio of $< 1: 30$, was observed (Table 1).

The Kaplan-Meier product limit method was used for analysis of remission duration patterns. For comparison of remission duration patterns of 2 or more groups, the logrank procedure was applied. All $p$ values reported refer to 2-sided tests.

Results

CFU-c Assay and Classification of Growth Patterns. The 3 CFU-c growth pattern categories used in this study were found...
The overall sensitivity for diagnosis of ANLL, however, is comparatively low, with only 55 of 96 patients (57%) exhibiting the pattern observed in any of the lymphoid neoplasias studied. This pattern appears to be quite specific for ANLL, with no AML-type growth observed in patients with ANLL (cf. Refs. 11 and 12) whenever it was clinically feasible and when adequate material was available. On the earlier L-12 protocol, when this comprehensive marker program was initiated, only 48 (46%) of a total of 104 protocol patients could be studied, while adequate bone marrow specimens could be analyzed in 48 of 58 patients (83%) treated on the subsequent L-14 protocol for ANLL.

**CFU-c Assay in the Diagnosis of ANLL.** Table 2 shows the CFU-c growth pattern (cf. Table 1) and the morphological diagnosis according to the modified FAB system (15) for 15 patients with ALL and 96 patients with ANLL studied at diagnosis. Since lymphoblasts do not grow under the CFU-c assay conditions (22), lymphoblastic leukemias either show no aggregate formation or residual colony and cluster growth of presumably normal myeloid progenitor cells (Table 2). None of these patients showed an AML-type growth pattern (cf. Table 1). Similarly, no AML growth patterns were observed in patients with chronic lymphoid leukemia and other lymphoid neoplasias with bone marrow involvement (22), further supporting the specificity of this pattern for ANLL. The AML growth pattern with low colony incidence and cluster excess was observed in 52 of 96 patients (54%) with ANLL. Three additional patients (3%) showed the high-cloning-efficiency AML growth pattern (cf. Table 1). Sixteen patients with ANLL (17%) showed a normal growth pattern, and the remaining 25 patients (26%) revealed no growth in culture. The highest incidence of no-growth and normal growth patterns was observed in acute undifferentiated leukemia (M0; 3 of 4), undifferentiated AML (M1; 3 of 3) and undifferentiated acute monocytic leukemia (M5a; 3 of 4). In contrast, a predominance of the AML-type growth pattern was seen in the partially differentiated acute myeloid (M2, 3; 21 of 29), in the partially differentiated acute monocytes (M5b; 12 of 22), and in acute myelomonocytic leukemia (M4; 5 of 7). The MDS including erythroleukemia (M6), RAEB, CMML, and RAEB-CMML progressing to ANLL exhibited growth patterns similar to those in patients with ANLL. The culture studies in MDS were obtained when these patients were started on protocol chemotherapy. Growth patterns during the chronic phase of MDS have been reported previously (14, 20). The current study includes only patients with MDS, who were started on protocol therapy because of blastic progression to ANLL (MDS → AL) or after becoming refractory to supportive therapy. The AML-type CFU-c growth pattern thus appears to be quite specific for ANLL, with no AML-type growth pattern observed in any of the lymphoid neoplasias studied. The overall sensitivity for diagnosis of ANLL, however, is comparatively low, with only 55 of 96 patients (57%) exhibiting the AML growth pattern in the total ANLL group. As described above, the sensitivity appears to be somewhat higher in the partially differentiated myeloid (M2, M3) categories, with 21 of 29 (72%) exhibiting an AML growth pattern.

**CFU-c Assay and Response to Therapy.** The CR rates by CFU-c growth pattern on the L-12 and L-14 protocols are shown in Table 3. On both protocols, the AML-type growth patterns were associated with the highest remission incidence with 64 and 81% CR, respectively. While 0 of 5 patients with a normal growth pattern achieved CR on the L-12 protocol, 7 of 11 patients with this feature obtained CR on the L-14 protocol, suggesting that the addition of the anthracycline drug on the L-14 protocol (2) was of special benefit for the group exhibiting a normal growth pattern in the CFU-c assay ($p = 0.03$). No significant differences were observed between CR rates obtained on both protocols in the other 2 growth pattern groups.

We have shown previously that partial differentiation by morphological criteria (M2, M3, M4, M5b) appears to indicate a somewhat better prognosis than is associated with the undifferentiated ANLL group (M0, M1, M5a) and the MDS group (M6, RAEB, CMML, MDS → AL) (15). Table 4 summarizes the CR rates by these morphological groups and their association with specific growth patterns. Although the numbers broken down by these categories become rather small, the AML-type growth pattern appears to be of prognostic significance for both, partially differentiating and the MDS group, while the growth pattern is without recognizable effect on CR rates in the undifferentiated ANLL group and also in ALL (Table 4). Although related to morphological class (see above; Table 2), the AML growth pattern thus appears to be of independent prognostic significance since its predictive value is seen in both partially differentiating ANLL and MDS.

### Table 1

**Definition of CFU-c growth patterns**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Colonies</th>
<th>Clusters</th>
<th>Colony:cluster ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>$&lt;5$</td>
<td>$&lt;5$</td>
<td>1:30</td>
</tr>
<tr>
<td>Normal</td>
<td>$&lt;50$</td>
<td>$&lt;1000$</td>
<td>&gt;1:30</td>
</tr>
<tr>
<td>AML type</td>
<td>$&lt;10^6$</td>
<td>$&gt;5$</td>
<td>&gt;1:30</td>
</tr>
</tbody>
</table>

* Three of 96 cases had high cloning efficiency with $>10$ colonies, but a ratio of 1:30.

### Table 2

**CFU-c growth pattern and modified FAB classification**

<table>
<thead>
<tr>
<th>Morphology</th>
<th>No growth</th>
<th>Normal</th>
<th>AML type</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL (L-1-L-3)</td>
<td>11 (37)</td>
<td>4 (27)</td>
<td>0 (0)</td>
<td>15 (100)</td>
</tr>
<tr>
<td>M0</td>
<td>0 (0)</td>
<td>3 (75)</td>
<td>1 (25)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>M1</td>
<td>2 (67)</td>
<td>1 (33)</td>
<td>0 (0)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>M2</td>
<td>3 (15)</td>
<td>3 (15)</td>
<td>14 (70)</td>
<td>20 (100)</td>
</tr>
<tr>
<td>M3</td>
<td>2 (22)</td>
<td>0 (0)</td>
<td>7 (78)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>M4</td>
<td>2 (28)</td>
<td>0 (0)</td>
<td>5 (71)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>M5a</td>
<td>2 (50)</td>
<td>1 (25)</td>
<td>1 (25)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>M5b</td>
<td>5 (23)</td>
<td>5 (23)</td>
<td>12 (55)</td>
<td>22 (100)</td>
</tr>
<tr>
<td>M6</td>
<td>1 (33)</td>
<td>0 (0)</td>
<td>2 (67)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>RAEB</td>
<td>1 (13)</td>
<td>1 (13)</td>
<td>3 (28)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>CMML</td>
<td>1 (14)</td>
<td>1 (14)</td>
<td>5 (72)</td>
<td>7 (100)</td>
</tr>
<tr>
<td>MDS → AL</td>
<td>3 (33)</td>
<td>1 (11)</td>
<td>5 (56)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>ANLL (total)</td>
<td>25 (26)</td>
<td>16 (17)</td>
<td>55 (57)</td>
<td>96 (100)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

### Table 3

**CFU-c growth pattern and CR rates by protocol**

<table>
<thead>
<tr>
<th>Pattern</th>
<th>No. /total</th>
<th>CR %</th>
<th>No. /total</th>
<th>CR %</th>
<th>No. /total</th>
<th>CR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0/5</td>
<td>0</td>
<td>7/11</td>
<td>64</td>
<td>7/16</td>
<td>44</td>
</tr>
<tr>
<td>No growth</td>
<td>7/15</td>
<td>47</td>
<td>3/10</td>
<td>30</td>
<td>10/25</td>
<td>40</td>
</tr>
<tr>
<td>AML type</td>
<td>18/28</td>
<td>64</td>
<td>22/27</td>
<td>81</td>
<td>40/55</td>
<td>73</td>
</tr>
<tr>
<td>Overall</td>
<td>25/48</td>
<td>52</td>
<td>32/48</td>
<td>67</td>
<td>57/96</td>
<td>59</td>
</tr>
</tbody>
</table>

* $p = 0.004$ for leukemic (40 of 55) versus nonleukemic (17 of 41).

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*CFL/-C Assay in ANLL*

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Unpublished observations.
In the recent morphological analysis of 263 adult patients with ANLL, a significant relationship was documented between the presence of Auer rods on bone marrow smears and both high CR rates and long remissions (15). We therefore analyzed the relationship between Auer rod distribution and growth pattern and their effect on CR rate (Table 5). Although the highest incidence of Auer rod-positive phenotypes was seen in the AML growth pattern group, the association of highest CR rate with AML group pattern was true for both the Auer rod-exhibiting and the Auer rod-negative groups (Table 5). The highest remission rate of 25 of 28 or 89% was observed in patients exhibiting both the AML-type growth pattern and Auer rods.

Initial CFU-c Growth Pattern and Remission Duration. Analysis of remission duration patterns by CFU-c growth category has failed to reveal significant relationships (Chart 1). Comparison of the AML pattern and "no growth-normal growth" groups according to the presence or absence of Auer rods reveals somewhat longer remission durations for the Auer rod-exhibiting patients in both growth pattern groups but again no recognizable effect of growth pattern on remission duration (Chart 2).

CFU-c Growth Patterns in Complete Remission. Previous studies had shown the association of AML-type growth patterns during clinical CR with subsequent relapse in individual cases (20, 26). In the current analysis, all CFU-c samples obtained from patients on the maintenance chemotherapy of the L-6 and L-12 protocols were analyzed. A total of 354 samples from 48 patients with ANLL was studied (Table 6). As a control group, 85 CFU-c assays were performed in bone marrow samples from 43 patients with ALL receiving maintenance chemotherapy on the L-10/L-10M protocols (Table 6). Using the criteria for classification of CFU-c patterns outlined in Table 1, no significant correlation of an AML-type growth pattern and subsequent relapse could be documented for ANLL. In most patients, marked fluctuations between CFU-c incidence and growth pattern were observed. In one patient with ANLL, 7 months after achieving CR and 5 months prior to relapse after 4 years in CR, a typical AML growth pattern was observed with very low colony formation and great excess of microclusters. These fluctuations in growth pattern appeared to be without relationship to chemotherapy cycles and could not be accounted for by technical variations. On both occasions, subsequent growth patterns returned to normal in this patient. Somewhat surprisingly, also some cases of ALL in CR on maintenance chemotherapy showed occasionally an AML-type growth pattern. Overall incidences of growth patterns in CR were not significantly different between ALL and ANLL. Similar to the observations in ANLL, no relationship between CFU-c growth pattern and subsequent relapse was recognized for ALL (Table 6).

Discussion

The definition of CFU-c growth patterns used in this study is a simplified version of the originally proposed growth pattern classification (20, 22, 26). The modification of the original classification proposed by Spitzer et al. (29) is quite similar.
with a further subdivision of the AML growth pattern category according to the absolute number of cell aggregates (29, 30). These authors combined the no-growth and normal variants into a "Group 1 (no growth)" category, while a total aggregate incidence of 1 to 20 was termed "Group 2" and one of >20 was termed "Group 3," if exhibiting a leukemic growth pattern, i.e., cluster excess. In the current study, subdivision of the group exhibiting the AML growth pattern into Group 2 and 3 categories according to the suggestion of Spitzer et al. (29) yields somewhat different distributions than reported by these authors. In this study, 45 (82%) of a total of 55 patients with leukemic growth pattern would fall into the Group 3 category as compared to 19 of 55 (35%) reported by Spitzer et al. (29). Whether these differences reflect technical variations of the CFU-c assay or are due to different patient populations remains to be analyzed. The overall incidence of AML-type ("leukemic") growth patterns was also found to be somewhat lower in the current study (57%) as compared to the results of Spitzer et al. (72%) (30) and to our own earlier observations (20, 26) but are somewhat different from those reported by Spitzer et al. (30). The Memorial Hospital group exhibiting the nonleukemic growth pattern exhibited a lower CR rate than reported by Spitzer et al. (30) for this group. On the other hand, the Group 3 pattern (29, 30) was associated with a much higher CR rate at Memorial Hospital. Technical differences between CFU-c assay systems, probably different patient populations as well as differences between chemotherapeutic regimens, could account for the observed discrepancies between results. In this study, we have not evaluated the CFU-c assay during induction chemotherapy as a predictive test for achieving CR as has been reported previously by Moore (20) and Spitzer et al. (30). In view of (a) the high CR rate on current chemotherapy protocols, (b) the difficulties in reproducible tissue sampling in severely hypoplastic patients, and (c) the lack of therapeutic consequences, we believed that this approach would provide little further information.

The combination of marrow culture and morphology allowed identification of subgroups of patients with high probability of achieving CR (Tables 4 and 5). It is of interest that morphological evidence for myeloid or monocytic differentiation and the presence of Auer rods were found to be associated with a good prognosis (15). Similarly, the CFU-c assay was related to high CR rates when showing functional evidence for myeloid differentiation, i.e., the ability to grow in soft agar in response to colony-stimulating factor.

The no-growth and normal-growth categories possibly reflect nonmyeloid ANLL, such as immature megakaryoblastic or erythroid equivalents which cannot be recognized by conventional morphological criteria (1, 3).

In contrast to the observations by Keating et al. (8), we have not detected a relationship between CFU-c growth pattern and remission duration (Charts 1 and 2). The disagreement between the results reported by Keating et al. (8) and our own observation might at least in part be due to the fact that the series analyzed by that group also included a relatively large proportion of patients with ALL.

Monitoring of the remission status by using sequential marrow culture studies during CR has been possible in individual cases (20). However, it has been very difficult in the majority of cases because of even spontaneous fluctuations in CFU-c incidence and growth pattern (4, 7, 31), which were also observed in this study. We have previously documented spontaneous shifts of growth patterns in MDS (14) as well as in CML,3 possibly indicating clonal expansion and regression (cf.
The Hematology/Lymphoma Service, Memorial Hospital, is appreciated. We acknowledge the significance of the CFU-c assay in comparison to other phenotypic markers, clinical and therapeutic parameters. The monitoring of the remission status, however, remains problematic due to spontaneous and therapy-induced variations of culture results.

Acknowledgments

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References


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