Autonomous Growth of Blast Cells Is Associated With Reduced Survival in Acute Myeloblastic Leukemia

By Ann E. Hunter, Stephen Y. Rogers, Irene A.G. Roberts, A. John Barrett, and Nigel Russell

We have previously classified the in vitro growth characteristics of clonogenic blasts from patients with acute myeloblastic leukemia (AML) according to their capacity to proliferate autonomously in a blast cell colony assay. Here we have analyzed whether the presence of in vitro autonomous growth characteristics has any clinical relevance in AML. We have studied 50 patients (age 2 to 64 years), all of whom were treated with combination chemotherapy, excluding patients with a history of antecedent myelodysplasia. Leukemic cells from 34 of 50 patients (68%) exhibited either partial or totally autonomous growth in a blast cell colony assay. Cells from the remaining patients exhibited nonautonomous growth and were either totally dependent on exogenous growth factor (n = 8) or failed to proliferate at all in the culture system used (n = 8). All 50 patients were treated by intensive chemotherapy and 69% achieved complete remission (CR). Patients whose blasts exhibited autonomous growth in vitro had a significantly lower CR rate (57%) compared with the 16 patients with nonautonomous growth (94%, P = .02). White blood cell count was the only other significant factor (P = .03), but in multivariate analysis growth characteristics remains the most important predictor of CR. Actuarial relapse risk is 80% and 42% at 5 years for autonomous and nonautonomous groups respectively (P = .1). Overall disease-free survival is 21.8% and is higher in the nonautonomous growth group at 54.2% compared with 11.3% at 5 years (P = .0015) in the autonomous growth group. Thus, the presence of autonomous growth characteristics was found to be the single most important indicator of CR and disease-free survival. Our data suggests that the suppression of autocrine growth factor production may be of value in the treatment of AML.

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A NUMBER of studies have shown that blasts from some patients with acute myeloblastic leukemia (AML) produce colony-stimulating factors (CSF) including granulocyte-macrophage CSF (GM-CSF), granulocyte-CSF (G-CSF), interleukin-1 (IL-1), and IL-6,1,2 and that leukemic cells that produce these CSFs frequently grow autonomously in clonogenic assays.3-10 We have previously reported that the blast cells from up to 70% of patients with AML exhibit either partially or totally autonomous growth in a clonogenic assay, the remainder either being growth-dependent on exogenous CSF or failing to grow in the culture system.7 It has also been shown that the autonomous growth of leukemic cells closely correlates with the production of CSFs including GM-CSF and IL-1β10 and that growth can be inhibited by neutralizing antibodies to these cytokines indicating that they are acting as autocrine growth factors.1,2,6,8,9 Until now the clinical significance of in vitro autonomous growth factor production has been uncertain. To address the clinical relevance of autocrine growth we have studied a series of 50 patients to ascertain whether growth characteristics have any predictive value as a determinant of treatment outcome in AML.

MATERIALS AND METHODS

The diagnosis of AML was established by morphologic review of May-Grünwald-Giemsa–stained smears of peripheral blood (PB) and bone marrow (BM). Classification of AML was made according to the French-American-British (FAB) criteria.1,2 Blood samples or BM for culture studies were obtained at diagnosis from 50 consecutive patients with de novo AML under the age of 65 years, excluding patients with a history of antecedent myelodysplasia. Other criteria for analysis were that all the patients had received treatment with chemotherapy protocols for remission induction that included an anthracycline and cytosine arabinoside according to Medical Research Council protocols (3 + 10 daunorubicin cytosine arabinoside thioguanine [DAT] or 2 + 7 DAT)12 or a regimen of daunorubicin, cytosine arabinoside, and lonustine (CCNU);13 and that all patients should have completed one cycle of chemotherapy, ie, early deaths were excluded from the study. All patients achieving complete remission (CR) were treated with at least two cycles of consolidation chemotherapy. In addition, 6 patients underwent BM transplantation (BMT) (5 allogeneic and 1 autologous), all in first complete remission. A complete remission was defined by a marrow with less than 5% blasts and normal morphology of hematopoiesis with normal PB counts. Overall survival was measured from diagnosis until death or last follow-up.

AML blast cell colony assay. The colony assay was performed as previously described.7 The leukemic cells were separated by Ficoll-Hypaque sedimentation (Pharmacia, Uppsala, Sweden) and then depleted of T lymphocytes before culture. For the blast cell colony assay, leukemic blasts were cultured at 2 × 10⁴ cells/mL in Iscove’s modified Dulbecco’s medium (IMDM) (Flow Laboratories, Irvine, UK) and 0.8% methylcellulose containing 10% fetal calf serum (FCS) (Flow Laboratories) in 96-well flat-bottomed microtiter plates (Costar, High Wycombe, UK), each well containing 2 × 10⁴ cells in 100 μL. Cultures were plated in triplicate in the presence or absence of a source of colony-stimulating activity provided by medium conditioned by the 5637-bladder carcinoma cell line (5637-CM) (10% final concentration), which is known to contain GM-CSF, IL-1. In some experiments recombinant GM-CSF (100 U/mL) or IL-3 (100 U/mL) (Genzyme, West Malling, UK) were also used. Colonies of greater than 20 cells were counted between 5 to 7 days of culture. For each patient the autostimulatory index (ASI) was calculated. This represents the number of colonies/2 × 10⁴ cells in the absence of 5637-CM divided by the number of
colonies/2 \times 10^4 \text{ cells in the presence of 5637-CM. Therefore, the ASI is a measure of the degree of autonomous growth of the leukemic cell population. }

Patients were classified into four groups, as previously described, dependent on the in vitro growth characteristics. Group 1 blasts failed to grow in this culture system, either autonomously or in response to 5637-CM, recombinant GM-CSF (rGM-CSF) or recombinant IL-3 (rIL3); group 2 blasts formed significant numbers of colonies (>10/2 \times 10^5 \text{ cells}) but only in the presence of 5637-CM (ASI < 0.1); group 3 blasts produced colonies in the absence of any form of CSFs but colony growth was further stimulated in the presence of 5637-CM, (ASI 0.1 to 0.8); group 4 blasts exhibited totally autonomous growth (ASI > 0.8), i.e. the number and size of colonies formed being virtually identical with or without the addition of CSF.

Statistical methods. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) (SPSS Inc, Chicago, IL). The relationship between each variable and growth characteristics was assessed using chi-squared and Fisher's exact probability test. Logistic regression analysis was used to assess the effect of the independent variables studied on CR, relapse, and overall disease-free survival. This was extended to multivariate analysis. Actuarial disease-free survival and relapse risks were estimated using the Kaplan-Meier technique and compared using the log-rank test.

RESULTS

Descriptive analysis. All 50 patients were included in the analysis. The growth characteristics of the blasts from these patients are shown in Table 1, 34 patients (68%) had evidence of autonomous growth (groups 3 and 4) and for further analysis these patients were grouped together (autonomous growth group) as were the 16 patients without autonomous growth (groups 1 and 2). Details comparing age, white blood cell (WBC) count, FAB type, and cytogenetics (obtained in 48 of 50 patients) of the two groups are shown in Table 2. Neither mean age nor WBC count at diagnosis were significantly different between the two groups and there was no significant difference in the FAB-type distribution or in the presence of unfavorable cytogenetics. Thus, the two groups were balanced with respect to other previously identified prognostic features.

Correlation of growth characteristics with clinical outcome. The overall CR rate for all patients in this study was 68%. Patients whose blasts exhibited nonautonomous growth had a significantly higher CR rate with 15 of 16 patients (94%) achieving CR compared with the autonomous growth group, where only 19 of 34 patients (57%) attained CR (P = .02). There was no statistical difference in the CR rate between group 3 (52%) and group 4 (61%) patients, thus validating our analysis of these patients as one group. We have analyzed the reasons for the low CR rate in the autonomous growth group. Of the 15 patients with autonomous growth who failed to achieve CR, 10 were considered to have resistant disease in that they did not achieve CR after two or more courses of remission induction chemotherapy; the remaining 5 patients died in aplasia after the first or second remission-induction course.

Other factors were analyzed in univariate analysis using logistic regression for their effect on CR (Table 3). Age, FAB type, or cytogenetics was not significantly associated with CR. Only the WBC count (analyzed as a continuous variable) reached statistical significance (P = .03) (Table 3). The lack of an effect of age on CR rate in this study is explained

<table>
<thead>
<tr>
<th>Table 1. Growth Characteristics of Blast Cells In Study</th>
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<tbody>
<tr>
<td><strong>No. of Patients</strong></td>
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<tr>
<td>Group 1</td>
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<tr>
<td>Group 2</td>
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<tr>
<td>Group 3</td>
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<td>Group 4</td>
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<tr>
<th>Table 2. Comparison of Clinical and Laboratory Features According to Growth Characteristics of AML Blasts In Vitro</th>
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<tr>
<td><strong>Nonautonomous Growth</strong></td>
</tr>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Age in yrs (mean)</td>
</tr>
<tr>
<td>(range)</td>
</tr>
<tr>
<td>WBC count, \times 10^9/L (mean)</td>
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<tr>
<td>(range)</td>
</tr>
<tr>
<td>Cytogenetics (%)</td>
</tr>
<tr>
<td>Favorable</td>
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<tr>
<td>Intermediate</td>
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<tr>
<td>Unfavorable</td>
</tr>
<tr>
<td>FAB (%)</td>
</tr>
<tr>
<td>M1</td>
</tr>
<tr>
<td>M2</td>
</tr>
<tr>
<td>M3</td>
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<tr>
<td>M4</td>
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<td>M5</td>
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<td>M6</td>
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<th>Table 3. Prognostic Factors Associated With the Achievement of Complete Remission</th>
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<tr>
<td><strong>Mean Age (yr)</strong></td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>No CR</td>
</tr>
<tr>
<td>Mean WBC count (\times 10^9/L)</td>
</tr>
<tr>
<td>CR</td>
</tr>
<tr>
<td>No CR</td>
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<tr>
<td>FAB type (% CR)</td>
</tr>
<tr>
<td>M1</td>
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<td>M2</td>
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<td>M3</td>
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<td>M4</td>
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<td>M5</td>
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<tr>
<td>M6</td>
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<tr>
<td>Cytogenetics (% CR)</td>
</tr>
<tr>
<td>Favorable</td>
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<tr>
<td>Intermediate</td>
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<tr>
<td>Unfavorable</td>
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<tr>
<td>Growth characteristics</td>
</tr>
<tr>
<td>Nonautonomous</td>
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<tr>
<td>Autonomous</td>
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by the fact that we confined this analysis to patients under 65 years. In a multiple linear logistic regression model, growth characteristics were the best predictor of achieving CR, with WBC count adding a small but significant contribution (Table 4).

We further analyzed the effect of growth characteristics on the relapse rate and overall disease-free survival. Of the patients who achieved CR, the relapse rates were significantly different between the two groups. Only 6 of 15 (40%) of patients in the nonautonomous growth group have relapsed compared with 14 of 19 (74%) in the autonomous group ($P = .05$). No patient has died while in CR. Of the 5 surviving patients in the autonomous growth group remaining in CR, 4 have undergone allogeneic BMT from an HLA-matched sibling, compared with 1 in the nonautonomous group. Age at diagnosis, WBC count, cytogenetics, and FAB type had no significant effect on relapse risk in this study.

Overall the actuarial disease-free survival for the 50 patients is 21.8% at 5 years, with a median survival of 8.4 months. Patients whose blasts exhibited nonautonomous growth have a statistically significant higher predicted survival of 54.2% at 5 years (median not reached), compared with the autonomous growth group who had a predicted survival of 11.3% at 5 years (median 6 months) ($P = .0015$) (Fig 1). In a logistic regression model, growth characteristics are the only significant predictor of disease-free survival (Table 5). In multivariate analysis, growth characteristics are the single most important factor with a small but significant contribution from the presenting WBC count (Table 6). These results mirror those for predicting clinical remission.

**DISCUSSION**

With current chemotherapy regimens, between 65% and 80% of patients with AML will enter complete remission and a subset of patients are cured of their disease.$^{1,10}$ A number of factors have been previously studied for prognostic value in AML. Despite the value of morphologic distinction between the various subtypes of AML there is no evidence that this has any prognostic value. In an analysis of patients entered into the Medical Research Council's 8th and 9th AML trials, FAB type was not of prognostic value. In the same clonogenic assay method was used for all patients using 5637-CM as a source of colony-stimulating activity. Leukemic cells were not depleted of adherent cells as this has not been found to affect the autonomous proliferation of AML cells in culture.$^{5,10}$ We confined this analysis to patients under the age of 65 years who had no history of antecedent myelodysplasia and who were treated with at least one course of intensive remission induction therapy, thus excluding patients with predetermined poor prognostic features. The overall CR rate for the whole group was 68%, which is comparable with recent major published series.$^{17,18}$ We found that patients whose leukemic cells exhibited autonomous growth (groups 3 and 4) had a significantly lower CR rate (57%) than patients with nonautonomous growth.

![Kaplan-Meier survival plots for patients whose blasts exhibit nonautonomous-growth (—) or autonomous-growth (==) survival was significantly different between the two groups ($P = .0015$, log-rank test).](https://www.bloodjournal.org/)

Table 4. Logistic Regression Model for Achieving Clinical Remission

<table>
<thead>
<tr>
<th>Regression</th>
<th>Coefficient</th>
<th>SE</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth group</td>
<td>1.378</td>
<td>0.6329</td>
<td>.0046</td>
</tr>
<tr>
<td>WBC count</td>
<td>-0.0137</td>
<td>0.0063</td>
<td>.0121</td>
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Table 5. Logistic Regression Analysis of Disease-Free Survival, Univariate Analysis

<table>
<thead>
<tr>
<th>Regression Coefficient (B)</th>
<th>SE</th>
<th>P Value</th>
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<tbody>
<tr>
<td>Age</td>
<td>0.222</td>
<td>0.222</td>
</tr>
<tr>
<td>WBC count</td>
<td>0.0169</td>
<td>0.0101</td>
</tr>
<tr>
<td>Growth group</td>
<td>-1.0046</td>
<td>0.3494</td>
</tr>
<tr>
<td>Cytogenetics</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>FAB type</td>
<td>—</td>
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gests a role for exogenous hematopoietic growth factors in
growth may be usefully incorporated into chemotherapy
reduced survival probability. Also, further evidence sug-
group is 11% at 5 years compared with greater than 50% for
the nonautonomous group.
A previous report by Preisler et al has suggested that the
expression of IL-1 mRNA by AML blasts is associated with
short remission duration. Our results are in agreement with
that data as we have previously shown that blast cell autono-
ous growth is closely related to autocrine or in some cases
paracrine production of GM-CSF and IL-1β. Why should
autonomous growth be associated with reduced CR rate and high risk of relapse in AML? On a theo-
etical basis one possibility is that the acquisition of autono-
mous growth characteristics may increase the inherent
aggressiveness of leukemic clones by making them independent of stromal cell production of CSF; however, other
mechanisms may be operative. Recently Koistinen et al reported that in 9 out of 10 cases, exogenous GM-CSF pro-
tected AML cells from cytokine arabinoside toxicity, com-
pared with G-CSF, leading to the suggestion that GM-CSF
treatment might reduce the cytotoxicity of cytokine arabin-
side by altering its intracellular metabolism. In contrast,
others have shown that both in vitro and in vivo, GM-CSF
can mobilize leukemic cells into 'S' phase which could theo-
etically overcome kinetic resistance to Ara C. However,
a recently reported clinical study showed that the addition of
recombinant GM-CSF to remission-induction treatment
for AML was associated with both a lower CR rate and a
reduced survival probability. Also, further evidence sug-
gests a role for exogenous hematopoietic growth factors in
inhibiting apoptosis of leukemic cells induced by cytotoxic
drugs. Although we have not studied the sensitivity of leu-
emic cells with different growth characteristics to cytotoxic
drugs, it is possible that autocrine growth factors may also
protect against cytokine arabinoside cytotoxicity.

Unlike other factors that have been shown to be of prog-
nostic significance in AML such as age, presenting WBC
count, and cytogenetic abnormalities, the biology of leuke-
mic cells is possibly open to therapeutic intervention. Our
findings that the autonomous growth of leukemic cells is
associated with reduced survival in AML would suggest that
the development of agents that suppress autonomous
growth may be usefully incorporated into chemotherapy
schedules. One possible approach is the use of recombinant
IL-1 receptor antagonist (IL-1ra), which has been shown to
suppress both autonomous growth and autocrine produc-
tion of GM-CSF and IL-1β. However, intracellular as well as extracellular autocrine loops appear to be operating in
AML cells; therefore, drugs that act intracelluarly to
suppress cytokine production may be of greater efficacy.

This study shows the value of in vitro growth characteris-
tics of AML blasts to subdivide AML patients into a good
prognostic group (>50% survival at 5 years), from a poor
prognostic group (<10% survival). The basis of this discrimi-
nation uses a simple clonogenic assay that provides quanti-
tative and qualitative information on the growth character-
istics of the leukemic cells within 5 to 7 days of culture; such
information could readily be incorporated into protocols
designed to treat AML patients according to risk. Of particu-
lar clinical value is the recognition of a subgroup of patients
(30%) with nonautonomous growth who have a relatively
good prognosis. However, this subgroup may be heteroge-
nous including as it does nongrowers as well as leukemias
with CSF-dependent growth; indeed some of the latter can
be induced to produce autocrine GM-CSF in response to
recombinant IL-1. It is possible that further studies of re-
sponses to different growth factors involving a larger series
of patients may yield further prognostic information.

ACKNOWLEDGMENT

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