The Sensitivity to Cytosine Arabinoside of the Blast Progenitors of Acute Myeloblastic Leukemia

By Nobuo Nara, J.E. Curtis, J.S. Senn, D.L. Tritchler, and E.A. McCulloch

Two culture methods are available for the study of the blast cells of acute myeloblastic leukemia (AML). One is an assay for clonogenic precursors; it depends on their ability to form blast colonies in culture in the presence of methylcellulose and suitable growth factors. The other assesses the growth of blast cells in suspension culture, where growth is measured by increasing numbers of colony-forming cells. We have compared the two methods as assays for the cytotoxic effects of the chemotherapeutic drug cytosine arabinoside (Ara-C). Marked patient-to-patient variation was found using either method; however, the slopes of the dose-response curves were usually greater when cells were exposed to drug in suspension rather than in methylcellulose. Control experiments showed that the difference could not be explained by drug carry-over from the suspension cultures to the methylcellulose plates when clonogenic cells in the suspensions were assessed. Further, the survival curves for Adriamycin were very similar, regardless of which assay was used. No correlation was found between $D_{10}$ Ara-C values measured in suspension or in methylcellulose. However, a significant association with outcome was found between $D_{10}$ Ara-C in suspension and response to treatment with a regimen in which Ara-C was the only chemotherapeutic agent used. No such association was detected when the $D_{10}$ values obtained with the clonogenic assay were compared with outcome for the same group of 15 patients. Finally, a feasibility experiment was performed in which blast cells were exposed to Ara-C repeatedly during exponential growth over 238 days. A dose-related inhibition of growth was observed; no evidence was seen of emerging drug-resistant cells. Nor did the morphology of the cells change as a result of drug exposure. We conclude that drug sensitivities of AML blast cells in culture are dependent on measurement methods, even when techniques affecting cell proliferation are compared. Measurements of drug sensitivity in culture may best be interpreted when the bases of the assay systems are understood.

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THE RESPONSE TO chemotherapy provides striking evidence of heterogeneity among patients with acute myeloblastic leukemia (AML). Some die before adequate treatment can be delivered. Many, but not all, treated patients enter remission. Of these, most relapse within a year, and only a few survive for 5 years. It is a research goal to understand the basis of this heterogeneity. We have approached the problem by seeking associations between outcome following chemotherapy and various attributes of patients, treatment regimens, or leukemic cells. For these last, cell culture methods have proved useful in the characterization.

We now employ two techniques for the culture of blasts. The first is a clonogenic assay: A small proportion of the blast population will form colonies in cultures made viscous by methylcellulose, provided a suitable source of growth factors is included. In the second method, blast progenitors multiply exponentially in suspension culture; the requirements for growth are a cell density of $10^6$ cells/mL and the same source of growth factors used in the clonogenic assay.

Both methods are consistent with a model of the blast population that postulates its maintenance by blast stem cells; these may renew themselves or enter a determination-like process yielding cells capable of only terminal divisions. In the clonogenic assay, colony formation reflects primarily these terminal divisions; self-renewal can be demonstrated only by replating colonies, either singly or as pools. This secondary plating efficiency (PE2), though variable from patient to patient, is always low, indicating that few of the divisions required for colony formation lead to new clonogenic cells. On the other hand, the exponential increase in blast progenitors seen in suspension cultures may be considered to reflect self-renewal; the presence of end cells is deduced from the observation that the doubling time in culture of blast progenitors growing in suspension is longer than their generation time. The difference in the extent of self-renewal seen in the two assays may reflect inhibitory effects of methylcellulose.

Blast heterogeneity is evident with either culture method. For the clonogenic assay, a significant association has been found repeatedly between successful remission induction and low plating efficiencies when blast colonies are subcultured (PE2). In contrast, no such link has emerged between response and the sensitivity of colony formation to the chemotherapeutic drugs used in treatment. Others have obtained similar results, particularly in settings where cytosine arabinoside (Ara-C) was the only chemotherapeutic agent used. It seemed possible that the effect of drug on self-renewal might be more important than its capacity to reduce colony formation. This view was strengthened with the finding that PE2 was reduced compared to controls when colonies surviving after exposure to Ara-C were replated, while no such change was found after exposure to Adriamycin.

These considerations led us to compare the Ara-C sensitivity of blasts when determined by each method. We found marked patient-to-patient variation regardless of technique; however, Ara-C was usually more effective against blasts in suspension than in methylcellulose. Further, the slopes of the simple negative exponential survival curves ($D_{10}$, dose required to reduce survival to 10% of control) obtained by...
each technique were not correlated; and for a small number of patients, a significant association was found between D0 Ara-C in suspension and successful remission induction. We also demonstrated the feasibility of exposing blast populations to drug repeatedly during prolonged exponential growth.

MATERIALS AND METHODS

Patients and treatment. Heparinized peripheral blood was obtained from 21 patients with AML at the time of diagnosis or at relapse, with informed consent, as prescribed by a protocol approved by the Human Experimentation Committee of the University of Toronto. The diagnosis was based on cell morphology according to the FAB classification and the clinical criteria used at the Ontario Cancer Institute in clinical trials of AML. Table 1 contains the clinical characteristics, treatment, and responses to treatment of the patient group. The patients seen at diagnosis received a high-dose Ara-C protocol. The protocol consists of Ara-C 3 g/m² of body surface area (BSA) twice for six days (reducing to 2 g/m² BSA in patients ≥60 years) and prednisone 40 mg/m² BSA daily for six days for remission induction. One patient (No. 6) refused treatment. Patient 5 had a previous neoplasm (carcinoma of lung). These two patients were excluded from the assessment of the response to therapy. The patients seen at relapse were treated with a combination of chemotherapeutic drugs.

Patients were considered to have responses if they had achieved either a complete remission (CR) or a partial remission (PR). The criteria for complete remission included a marrow of approximately normal cellularity with <5% blasts and <5% promyelocytes, normal number of megakaryocytes, and evidence of regenerating granulopoiesis and erythropoiesis. Normal peripheral blood counts and no evidence of clinical disease elsewhere were also required. A partial remission required a marrow of normal or moderately increased cellularity with >10% of blasts and promyelocytes but <25% blasts present. Patients were followed by examinations of the peripheral blood; in all instances, the blast population was eliminated. Early (14-day) marrow aspirations were not done routinely since, in our hands, this procedure has not proved useful clinically. Bone marrow was aspirated to assess response to treatment when normal leukocytes or blast cells began to appear in the peripheral blood. This usually occurred 4 to 5 weeks after treatment was started.

Blast colony assay. Primary colony formation was obtained as previously reported, except that supernatants from cultures of the continuous line of bladder cancer cells, HTB9, obtained from the American-type culture collection, were used rather than media conditioned by leukocytes in the presence of phytohemagglutinin (PHA-LCM). Suspensions containing 4 × 10⁶ T cell-depleted mononuclear cells from peripheral blood suspended in 1 mL of α-MEM supplemented with 20% fetal calf serum (FCS), 10% HTB9-conditioned medium, and 8% methylcellulose were cultured in 35-mm Lux culture dishes (Miles Laboratories, Naperville, Ill) for seven days at 37°C and 5% CO₂ in air. Colonies with more than 20 cells were scored, and plating efficiency was described as PE₁. Four replicate plates were used to determine PE₂ for each assay.

Replating from methylcellulose. Blast progenitors growing in methylcellulose were replated as described previously, except for the substitution of HTB9-conditioned medium for PHA-LCM. Cell suspensions prepared from primary blast colonies in methylcellulose were washed twice in α-MEM and then replated at a concentration of 10⁶ cells in 1 mL in Linbro microwells (Flow Laboratories, McLean, Va) of α-MEM with 20% FCS, 10% HTB9-conditioned medium, and 8% methylcellulose. Colonies with more than 20 cells were scored after seven days' incubation, and their plating efficiency was designated as PE₂. Four replicate wells were used to determine PE₃. Secondary colonies have been shown to be similar to primaries in terms of growth requirements and cellular morphology by light and electron microscopy.

Liquid suspension culture. Blast progenitors were cultured in liquid suspension as described previously, except for the substitution of HTB9-conditioned medium for PHA-LCM. T cell-depleted mononuclear cells from peripheral blood at a concentration of 10⁶/mL were suspended in 3 mL of α-MEM supplemented with 20% FCS and 10% media conditioned by HTB9 cells. The blasts were cultured in suspension in 35-mm Lux tissue culture dishes at 37°C in a humidified atmosphere of 5% CO₂ in air. Cultured cells were harvested and counted at day 7. Primary and secondary plating efficiencies (PE₁ and PE₂) were determined using the methylcellulose culture method described above.

Assessment of blast progenitor growth in suspension. The growth of clonogenic blasts in suspension was assessed by combining the methylcellulose assay with the suspension culture assay. After a period of time in suspension (usually seven days), the cells were harvested, washed, counted, and plated in methylcellulose to obtain the plating efficiency. By multiplying the number of cells recovered from each dish by this plating efficiency, the number of clonogenic cells per dish was calculated. This value could be compared to the input of nucleated cells (see Figure 2, for example). For experiments where cells were replated serially, the dilution at each subculture could be used to calculate the cumulative growth of clonogenic blast cells with time (for an example, see Fig 8).

Sensitivity to Ara-C. Figure 1 illustrates the method used to measure the sensitivity of blast progenitors to Ara-C in suspension or in methylcellulose. Blast cells were placed in either culture condition

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis (FAB)</th>
<th>Age</th>
<th>Peripheral Blood Blasts per μL</th>
<th>Treatment*</th>
<th>Outcome†</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M2</td>
<td>29</td>
<td>5,400</td>
<td>HD-Ara-C</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
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<td>25,600</td>
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<tr>
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<tr>
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<td>27</td>
<td>3,706</td>
<td>HD-Ara-C</td>
<td>F</td>
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</table>

*HD-Ara-C, high-dose cytosine arabinoside; ACT, Adriamycin, Ara-C, and 6-thioguanine; L-asp, L-asparaginase; MTX, methotrexate.
†PR, partial remission; CR, complete remission; F, failure; PC, previous cancer (not included in assessment; see Materials and Methods; NA, not applicable.)
Fig 1. How drug dose-response curves are obtained for AML blast progenitors exposed to drug for seven days in either suspension culture or methylcellulose culture.

for seven days in the presence of increasing concentrations of Ara-C. At the end of the period, the suspension cultures were harvested, washed twice in α-MEM, and assayed for colony formation in methylcellulose in the absence of Ara-C. The recovery of clonogenic cells per dish was calculated as described above, and its decrease with increasing exposure to Ara-C in suspension was used to construct survival curves. After seven days, the colonies in the methylcellulose cultures in the presence of drug were counted; the plating efficiency decreased as a function of drug dose, as described previously. Thus two survival curves were obtained for the same cell population exposed to Ara-C for the same time period, but either in suspension or in methylcellulose.

Sensitivity to Adriamycin. Similarly, the sensitivity of blast progenitors to Adriamycin was measured in suspension or methylcellulose cultures. Two regimens were used. In the first, the cells were exposed to varying concentrations of drug for ten minutes, washed, and cultured either in methylcellulose or in suspension. In the second, the cells were exposed continuously to the Adriamycin during seven days' growth in methylcellulose or suspension cultures. In the latter instance, the cultures were washed before replating the cells in methylcellulose in the absence of Adriamycin.

Cellular phenotypes. Cellular phenotypes were determined by using several monoclonal antibodies. Among them, OKM-1, 80H.5, and My-1 were used as reagents specific to granulopoietic cells. MO-2 was used as a reagent specific to monocytes.

Statistics. Group data were compared by Student's t test. D0 values for Ara-C and Adriamycin were determined from the slope of negative exponential dose-response curves calculated by linear regression. Dose-response curves were compared as described by Gaddum. Data are presented as the mean ± SE. Jonckheere's test was done as described in Lehmann.

RESULTS

Ara-C survival curves. Quantitative measurement of survival curves depends on the relationship between the number of cells added to cultures and the outcome of the procedure. Figure 2, representative of two experiments using samples containing sufficient blasts, shows this relationship to be linear for the number of blasts cells used to initiate suspension cultures and the recovery of clonogenic cells after seven days (r = .971, P < .01). Further, the presence of Ara-C in the cultures, while decreasing the yield of clonogenic blasts, did not alter the linearity of the relationship between blast input and clonogenic blast recovery (P < .05).

A typical Ara-C survival curve for blasts in suspension is shown in Fig 3. The simple negative exponential form of the curve is apparent. The survival curve obtained using the clonogenic assay, as described in Materials and Methods, is included in the figure. As reported previously, such survival curves are also simple negative exponentials. Both results can therefore be described by a single parameter, the D10. It is evident in Fig 3 that the D10 value was much greater for the clonogenic assay than for the suspension culture procedure.

The D10 values obtained for both methods are compared in
Fig 4. No significant correlation was found between the two measurements ($r = .249$, $P > .25$). It is evident that the Ara-C survival curve measured in suspension was usually less than that obtained using the clonogenic assay. In one instance, patient 17, $D_{10}$ in suspension was slightly greater than $D_{10}$ in methylcellulose.

**Drug carry-over control.** In the suspension culture methods, Ara-C is added to cultures at varying concentrations; after seven days, the cells are washed and replated in methylcellulose. It is possible, therefore, that Ara-C itself or an active metabolite might be carried from the suspension cultures into the methylcellulose assay plates. The action of such carried-over drug might explain the marked sensitivity observed with the suspension culture method.

Two experimental designs were used to test for this possibility. In the first, blast cells were exposed to Ara-C for seven days, recovered, washed, and irradiated to destroy their proliferative capacity. These were then added to varying numbers of intact cells, and the mixture was plated in methylcellulose. Colony formation as a function of the number of intact cells plated is shown in Fig 5A. It is evident that the plating efficiencies of clonogenic blasts in methylcellulose had not decreased when plated in the presence of cells that had been exposed to Ara-C under the conditions used to measure drug sensitivity in suspension.

In the second control design, irradiated cells obtained from cultures with or without Ara-C were added to varying numbers of intact cells, and the mixtures were cultured in suspension. Figure 5B shows the results obtained when the cells were tested for clonogenic blasts after seven days. The same linear relationship between input viable blasts and recovery of clonogenic blasts was maintained regardless of whether the irradiated supporting cells had been exposed to Ara-C or not.

These two experiments make carry-over of Ara-C an unlikely explanation for the differences in Ara-C survival curves seen when blasts were tested in suspension or in methylcellulose. In addition, the second design shows that exposure of blasts to Ara-C does not destroy their capacity to support the growth of small numbers of intact cells in suspension.

**Adriamycin sensitivity.** The comparison between Ara-C survival curves using two different methods is based on the assumption that both examine the same proliferative entity. The requirement for a single growth unit in each is supported by the linear relationship between cells added to cultures and response shown for each method in Fig 2 and 5. Evidence has been presented that the two procedures display different properties of the same cell. However, the possibility remains that a cell more primitive than the clonogenic blast detected in methylcellulose is growing in suspension and then undergoes a transition to a clonogenic blast when placed in methylcellulose. The existence of such a blast progenitor might explain the different Ara-C survival curves found in suspension and in methylcellulose. If this were the explanation, survival curves for a different drug might be expected to show the same phenomenon. Adriamycin is a candidate since marked patient-to-patient variation is found when Adriamycin treatment curves are measured. Further, Adriamycin differs from Ara-C in its effects on the composition of colonies surviving drug exposure. Colonies in Ara-C-treated plates often contain fewer new clonogenic cells than controls; in contrast, the PF2 values for colonies developing after Adriamycin treatment are similar to control values. These data have been interpreted as indicating that cell-cycle processes required for self-renewal might be more sensitive to Ara-C than those that serve cell division generally.

Blast progenitor survival curves for Adriamycin are shown in Fig 6. In the experiment, either cell suspensions were exposed briefly to drug, or Adriamycin was included in the culture medium. It is apparent that much greater sensitivities were found with the latter procedure. However, regardless of the length of exposure of cells to Adriamycin, very

![Fig 4. Relationship between $D_{10}$ values in suspension and in methylcellulose cultures. There is no significant correlation ($P > .2$). $r = 0.249.$](image-url)
similar D_{10} values were obtained both with the methylcellulose procedure and after growth in suspension. This finding supports the view that the growth unit is the same or very similar in both the suspension and methylcellulose assays and strengthens the basis for comparing survival curves obtained by the two methods.

**Association with outcome.** In our previous studies, D_{10} Ara-C measured in methylcellulose was not an attribute contributing to variation in outcome.\(^{10}\) This conclusion was based on clinical data from patients treated with multidrug regimens and using the assay for colony formation in methylcellulose. Although sensitivity data are available for only 15 patients treated with high-dose Ara-C at the time of first diagnosis, we undertook to compare the association of successful remission induction with D_{10} values measured either in suspension or in methylcellulose (Fig 7). When we followed our usual practice (see Materials and Methods) by including in the analysis all patients who received at least one dose of drug and by considering partial remissions as responses, a significant association (\(P < .01\)) (see Materials and Methods) was found for D_{10} values in suspension but not in the 14 samples assayed in methylcellulose (\(P > .2\)). Additional analyses were done after removal from the data set of patients who died within the first 48 hours of treatment. In the first of these analyses partial remissions were not included; significant association was maintained between low D_{10} values in suspension culture and successful remission induction (\(P < .05\)) but not for the methylcellulose assay (\(P > .2\)). Only if the two patients with partial remission were considered as failures did the association of D_{10} value in suspension with remission induction lose significance. It seems inappropriate to add the partially responding patients to the failure group, since, following treatment with Ara-C, blasts were reduced in these patients and normal hematopoiesis improved. It may be more useful to group the patients is three sets: remission, partial remission, and failure (excluding early deaths). Then the D_{10} values for each set can be compared using Jonckheere's test,\(^{27}\) which is the appropriate statistic to determine if sets are ordered in ascending values of D_{10} (that is, D_{10} CR < D_{10} PR < D_{10} failure). This analysis showed that such ranking is significant for these data (\(P < .05\)). This finding is consistent with the hypothesis that increasing D_{10} values in suspension are associated with decreasing probability of responding to treatment. Jonckheere's test did not give significant \(P\) values when applied to the D_{10} values measured in methylcellulose.

We considered other factors that have been found to be associated with outcome. In Table 2, age, number of blasts per milliliter in the peripheral blood, platelet count, and plating efficiencies in methylcellulose (PE, and PE2) are compared with D_{10} Ara-C in suspension; the results of testing these attributes for an association with successful remission induction are included in the table. As in previous studies,\(^{8,10}\) a significant association was found between PE and response; PE2 was also correlated with D_{10} Ara-C in suspension. None of the other characteristics of patients or of their disease were associated with outcome of treatment or with D_{10} Ara-C in suspension. These patients were all treated with large doses of Ara-C (see Materials and Methods); not surprisingly, blasts were cleared from the peripheral blood in all patients who received a full initial course of treatment, including those who failed to respond (Table 3). It may be, therefore, that the association between low D_{10} Ara-C values, measured in suspension, and successful remission induction is based on some drug action in addition to general cytotoxicity.

**Repeated exposures to Ara-C.** A major advantage of the suspension culture is that some blast populations may be

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**Table 2.** Statistical Associations of Attributes

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Association With Remission Induction</th>
<th>Association With D_{10} Ara-C in Suspension Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Association</td>
<td>(P)</td>
</tr>
<tr>
<td>Age</td>
<td>&gt; .1</td>
<td>.030</td>
</tr>
<tr>
<td>Blasts in blood</td>
<td>&gt; .1</td>
<td>.272</td>
</tr>
<tr>
<td>Platelets</td>
<td>&gt; .1</td>
<td>.002</td>
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<td>PE_{1}</td>
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<td>.135</td>
</tr>
<tr>
<td>PE_{2}</td>
<td>&lt; .01</td>
<td>.503</td>
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</table>
maintained in the culture for periods of weeks. This permits the examination of the effects of repeated exposures to chemotherapeutic agents, a setting that mimics the clinical use of drugs. Figure 8 displays the results of an experiment in which blast cells were exposed to Ara-C repeatedly over a period of 238 days. Two doses were used, $10^{-7}$ and $10^{-6}$ mol/L; the Ara-C was added at the time of subculture, and the cells were not washed until the next scheduled change of medium, 1 week later. Thus the drug exposure schedules were the same as those used to measure Ara-C survival curves.

It is evident that the control cultures grew exponentially, with a doubling time of between three and four days. At the lower drug dose, growth was inhibited immediately following exposure but resumed after the next subculture. In the cultures that received Ara-C at the $10^{-6}$ mol/L dose, a fall in clonogenic cells was seen after each exposure, followed by a resumption of growth after the cells were washed and resuspended.

We looked for changes in blast cell properties following repeated drug exposure. At the high dose, the degree of fall in clonogenic blasts was similar following each exposure, indicating that sensitivity to Ara-C had not changed. Sufficient cells were available from the control cultures and those exposed to $10^{-7}$ mol/L Ara-C to permit the construction of detailed survival curves. After 96 days in culture, the $D_{90}$ values were $2.4 \times 10^4$ and $3.4 \times 10^4$ mol/L for the control and drug-exposed cultures, respectively. These values are not significantly different ($P > .05$). At day 189, the blast progenitors remained Ara-C-sensitive, with a $D_{90}$ for the $10^{-7}$ mol/L Ara-C-treated cultures of $1.5 \times 10^4$ mol/L. The morphology of the cells remained unchanged throughout; in excess of 70% were typical blast cells; the remainder showed some granulation and occasional segmented forms were seen. The predominant immunologically defined markers on the blasts were those associated with granulopoietic cells and macrophages (OKM-1, 8OH.5, My-1, and MO-2); their frequencies did not change during long-term culture, nor were there differences in morphology or marker-defined phenotype between control and Ara-C-treated blast populations.

After 10 weeks in culture, the growth requirements were retested; the need for HTB9-conditioned medium and a cell density of $10^6$ cells/mL had not changed. We conclude that in this experiment, repeated exposures to Ara-C did not lead to drug resistance or other phenotypic changes in the blast population.

### Table 3. Failure Group

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Aplasia of Bone Marrow</th>
<th>Clearance of Blasts From Blood</th>
<th>Blast Regrowth in Bone Marrow</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>ND</td>
<td>NA</td>
<td>ND</td>
<td>Died at 1 day</td>
</tr>
<tr>
<td>7</td>
<td>no</td>
<td>yes</td>
<td>ND</td>
<td>Died at 1 month</td>
</tr>
<tr>
<td>9</td>
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<td>yes</td>
<td>yes</td>
<td>Died at 1 year</td>
</tr>
<tr>
<td>13</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
<td>Died at 4 months</td>
</tr>
<tr>
<td>15</td>
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<td>Died at 1 day</td>
</tr>
<tr>
<td>17</td>
<td>ND</td>
<td>yes</td>
<td>ND</td>
<td>Died at 2 months</td>
</tr>
</tbody>
</table>

NA, not applicable; ND, not determined.

*Blasts were predominant in a cellular bone marrow 4 weeks after treatment, but no further assessment was conducted before death.
their actions on cells; Adriamycin may reduce cell prolifera-
tion generally, while Ara-C may have additional toxicity for
genetic mechanisms required during self-renewal. From this
view point, Adriamycin dose-response curves might be
expected to be similar in any assay that depended on cell
proliferation, while Ara-C might appear more toxic in an
assay that reflects self-renewal.

This hypothesis may be considered in light of the associa-
tion of blast properties with remission induction. Low values
of PE2 have been correlated repeatedly with successful
remission induction.5,9,10 Although the small number of
patients available for analysis limits the power of the statisti-
cal tests, an association is emerging between low Ara-C D10
values in suspension and success of treatment with Ara-C.
In contrast, for the same series of patients, analyzed by the
same statistical methods, no association with response was
found when drug sensitivity was measured using colony
formation in methylcellulose as an assay. It seems unlikely
that drug cytotoxicity in itself would explain an association
between response and sensitivity in suspension but not in
methylcellulose. The doses of Ara-C administered to the
patients were high enough to ensure that blasts were cleared
from the peripheral blood even in those failing treatment,
making it improbable that insufficient cell kill explained
failure to achieve remission in the four failing patients who
received adequate courses of Ara-C. Rather, sensitivity in
suspension may improve response not only through general
cytotoxicity but also by interacting with some other compo-
ent of blast cell function. It is attractive to suggest that
this function might be self-renewal. Then the associations with
successful treatment of both low PE2 and sensitivity to Ara-C
in suspension might have a similar basis. A large prospective
study is needed to evaluate the contributions of PE2 and
Ara-C sensitivity in suspension, using multivariate methods.

We have demonstrated the feasibility of exposing blast
cells to chemotherapy repeatedly. The data of Fig 8 support a
simple cytotoxic model of chemotherapy. At low doses, the
doubling time of the blasts was prolonged; at a high dose,
reduction of clonogenic blasts was seen after each exposure,
followed by regrowth and eventually cessation of prolifera-
tion. For this dose, the extent of the reduction was similar
after each exposure, indicating that Ara-C sensitivity had
not changed. At the lower dose, sufficient cells were available
to construct survival curves. The D10 values were not signifi-
cantly different (P > .05). Thus we did not obtain evidence of
the emergence of drug-resistant cells. The morphology and
immunologically defined phenotypes of the blast population
remained similar in the treated and control groups. No
evidence of increased differentiation was seen. It is noted,
however, that this experiment, though requiring a massive
effort over 238 days, is but a single example. It needs to be
repeated with other blast populations and using other sched-
ules. Our results show only that cells in culture can be used to
model the repeated drug exposures used in treatment.

Finally, a major conclusion from these data has certain
consequences for the assessment of chemotherapy using cell
culture methods. It is evident that the dose-response curve
for any agent may be dependent on the method used to
determine it, even when techniques are compared that reflect
drug effects on cell proliferation. It follows that the use of
such dose-response curves, whether for predicting outcome of
treatment or selecting drugs, will be most effective if the
mechanisms underlying the assay procedures are under-
stood.

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