Characteristics of Cell Proliferation in Acute Leukemia[1]

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SUMMARY

Bone marrow aspirates and venous blood samples from 26 subjects with acute leukemia in relapse and from 10 nonleukemic controls were incubated *in vitro* with tritiated thymidine and examined by autoradiography. Twenty leukemic patients were studied again during the course of chemotherapy. The number of labeled cells per 100 nonerythroid nucleated cells provided an estimate of the population, which was in the DNA-synthesis phase of the generative cycle. This ratio was used as an indicator of the size of the proliferative population of bone marrow. The fraction of proliferating cells was significantly smaller in the leukemic bone marrows during relapse than in the controls. In the leukemic subjects, the peripheral blood blasts showed a smaller ratio of proliferating cells than that in bone marrow, but this difference was statistically not significant. The 13 patients who eventually responded to chemotherapy showed a marked increase in the actively proliferating population in the bone marrow early in the course of therapy, whereas all those patients who failed to go into remission showed no early rise in the proliferating pool. This procedure may assist in the early detection of nonresponders to the cytocidal agents with which acute leukemia patients are being treated so that it may be possible to change the therapeutic approach before toxicity ensues.

INTRODUCTION

A quantitative study of mammalian cell proliferation has become possible with the use of TdR-[3]H[3] as a DNA marker. We have studied the proliferative potential of the leukemic cells in patients with acute leukemia before and during therapy with cytocidal drugs and have attempted to correlate this with the eventual response to the chemotherapy. Blood and bone marrow cells incubated *in vitro* with TdR-^{3}H were examined by autoradiography to determine the ratio of TdR-^{3}H-labeled cells as an index of the proliferative cell population.

MATERIALS AND METHODS

Eleven patients (10 children and 1 adult) with acute lymphoblastic leukemia in relapse were studied before and during therapy. Of these 11, 7 were previously untreated and 4

were in their 2nd relapse. Fifteen adults with AML in relapse were studied prior to initiation of therapy. Of this group, 13 were previously untreated and 2 were in their 2nd relapse. Of these 15 patients, 9 were studied again during the course of therapy. Ten patients with nonleukemic diseases were studied as control. These patients were subjected to bone marrow aspirations in the course of hematological consultation, and no evidence of leukemia or other malignant conditions was found.

Bone marrow aspirate, 0.1 ml, was mixed with 5 ml Culture Medium 1-A (Grand Island Biological Co., Grand Island, N. Y.) in a sterile test tube, and 5 μCi of TdR-[3]H (New England Nuclear, Boston, Mass.; specific activity, 6.7 Ci/mM) was added. The gross number of cells ranged from 5 to 8×10^6. When peripheral blood was used for labeling, the leukocyte count was adjusted to the same range as that used for bone marrow preparations. The cell suspension was incubated at 37° for 2 hr. At the end of incubation, the cells were sedimented by centrifugation at 800 rpm for 10 min, and the supernatant was discarded. The cells were washed once with 0.9% NaCl solution and were smeared on gelatin-coated glass slides. Each study was conducted in duplicate, and 5 smears were made from each culture tube. Cell morphology and adequacy of number of cells were checked on slides treated with Wright's stain. For autoradiography, the slides were fixed in Carnoy's solution for 10 min, washed in tap water for 2 hr, and dipped in Eastman Kodak NTB-2 emulsion. An exposure time of 6 days was used. The background over the nonlabeled cells was less than 3 grains, and a cell containing 5 or more grains was considered labeled. Most of the labeled cells contained at least 20 grains, so there was no difficulty in distinguishing them from unlabeled cells. For calculation of LI (%), the nucleated cells belonging to erythroid series were excluded, and a minimum of 2500 cells with granulocytic and lymphocytic characteristics were counted on each sample.

All patients with leukemia were started on therapy as indicated below after the initial bone marrow and peripheral blood studies with TdR-^{3}H had been performed. Vincristine and prednisone were given to patients with acute lymphoblastic leukemia, while those with AML were given either (*a*) daunorubicin, (*b*) cytosine arabinoside, or (*c*) vincristine + 6-mercaptopurine + methotrexate + prednisone. Twenty patients were followed during and after therapy. After about 3 weeks of therapy, the studies were repeated for TdR-^{3}H LI of bone marrow and blood, and those patients who eventually responded and went into remission were studied for the 3rd time while in remission.

RESULTS

The data in Table 1 show the LI of bone marrow and peripheral blood of all the subjects studied prior to initiation

Table 1

TdR-³H in vitro LI of bone marrow and peripheral blood in acute leukemia

| Patient | Bone marrow LI (%) | | | Peripheral blood LI (%) | |
	Control	AML[a]	Acute lymphoblastic leukemia[a]	Acute lymphoblastic leukemia[a]	AML[a]
1	18.0	0.8	2.0	1.4	0.0
2	20.0	1.2	2.5	5.6	1.0
3	20.0	4.3	5.0	1.4	2.0
4	21.4	5.2	10.5	ND[b]	1.0
5	26.4	6.4	11.0	28.0	ND
6	28.0	6.6	11.0	ND	4.0
7	30.0	11.5	11.3	10.5	4.7
8	31.2	11.8	11.3	3.0	6.0
9	33.6	12.0	18.0	5.0	ND
10	40.0	13.2	18.1	15.1	3.8
11		13.3	22.6	ND	7.0
12		14.1			9.0
13		⁄ 15.6			ND
14		19.0			ND
15		22.3			4.7
LI (%)	26.9 ± 6.7[c]	10.5 ± 6.1	11.2 ± 6.2	8.8 ± 8.4	3.9 ± 2.7

[a] In relapse.
[b] ND, not done.
[c] Mean ± S.D.

Table 2

In vitro TdR-³H LI of bone marrow cells in patients with acute leukemia; before, during, and following therapy

| Diagnosis[a] | Response to therapy | LI during relapse (%) | | | LI after 3 weeks of therapy (%) | | | LI during remission (%) | | |
		Range	Mean	±S.D.	Range	Mean	±S.D.	Range	Mean	±S.D.
Acute lymphoblastic leukemia (8)	Good	2.0–22.6	11.4	6.2	40.0–42.1	41.3	0.0	20.0–38.0	26.0	5.2
AML (5)	Good	4.3–22.3	14.0	5.8	30.0–44.0	36.0	5.7	19.5–38.5	27.5	5.3
Acute lymphoblastic leukemia (3)	Poor	2.5–18.1	10.6	6.4	2.4–10.6	6.3	3.3	No remission		
AML (4)	Poor	1.2–11.8	7.7	4.4	0.0–14.6	5.4	5.6	No remission		

[a] No. of patients studied is given in parentheses.

of therapy. The mean LI of 10 control bone marrows was 26.9 ± 6.7%, which was significantly greater than the mean LI (11.2 ± 6.2% and 10.5 ± 6.1%) of 11 acute lymphoblastic leukemia and 15 AML bone marrows, respectively, with $p < 0.01$ by χ^2. There was no significant difference ($p > 0.5$) between the mean LI's of bone marrows in acute lymphoblastic leukemia and AML. The peripheral blood from the control subjects showed no cells labeled with TdR-³H. The difference between the mean LI's of peripheral blood in acute lymphoblastic leukemia and AML was not significant ($p > 0.3$). In AML, the peripheral blood cells showed a somewhat lower mean LI than did the bone marrow, but the difference was not statistically significant ($p > 0.2$), and in acute lymphoblastic leukemia the difference was even less impressive.

Of the 20 leukemic patients in whom studies were repeated during the course of antileukemia chemotherapy, 13 responded favorably and went into remission; the nonresponding 7 patients died within 2 to 3 months after the initial studies. At the time of repeat studies with TdR-³H (almost 3 weeks after the initiation of chemotherapy), the differential pattern of bone marrow of all patients was still predominantly blastic and relatively unchanged from the

original bone marrow of relapse. Although at this stage these patients showed some improvement in clinical status, including peripheral blood picture and subjective findings, these criteria were not adequate to predict whether a patient was likely to go into remission on continued therapy. However, as can be seen in Table 2, the 13 patients (8 with acute lymphoblastic leukemia, Patients 1, 3 to 7, 9, and 11 of Table 1; and 5 with AML, Patients 3, 10, 12, 13, and 15 in Table 1) who eventually responded showed a significant increase in the bone marrow TdR-³H LI ($p < 0.01$ by χ^2) after 3 weeks of therapy. In contrast, the 7 patients (3 with acute lymphoblastic leukemia, Patients 2, 8, and 10 in Table 1; and 4 with AML, Patients 2, 5, 7, and 8 in Table 1) who failed to respond to therapy showed a slight decrease in the bone marrow LI after 3 weeks of therapy, as compared to the pretherapy study, but the difference was not significant ($p > 0.5$). The TdR-³H *in vitro* bone marrow labeling was studied for the 3rd time (during the stage of remission) among the 13 subjects who responded to chemotherapy and was found to be similar to the LI of the bone marrow of the control subjects. The peripheral blood showed no labeled cells in any of the 20 patients studied following the institution of therapy.

DISCUSSION

The currently available morphological criteria do not distinguish between a normal myeloblast or lymphoblast and its leukemic counterpart, but because the preponderant cell in the bone marrow of a patient with AML or acute lymphoblastic leukemia in relapse is a blast, the latter is generally termed a leukemic blast cell. The studies (Table 1) that use TdR-^{3}H labeling as an index of cells in active generative cycle clearly confirm (3, 8, 11) that, contrary to belief, the majority of leukemic blast cells in the bone marrow of patients with acute leukemia are in a quiescent or nonproliferative stage, and nearly 80% of the blast population of bone marrow may be nonproliferative. It has also been observed (7, 10) that the fraction of actively proliferating blasts in the peripheral blood is even smaller than that present in the bone marrow in acute leukemia. The peripheral blood TdR-^{3}H LI in our series of patients was generally lower than the bone marrow LI, but this difference was not statistically significant.

Recent investigations (2, 12) indicate that the leukemic blast cells that are not in active cycle are not all end-stage cells, but at least some of these may potentially reenter the active generative cycle. These observations are significant for therapeutic considerations because most chemotherapeutic agents used in acute leukemia act by interference with the generative cycle. In other words, the cells not in active cycle escape the cytocidal effect of the drug, and this may explain some of the difficulties inherent in the management of acute leukemia. From the data reported in this paper, it is clear that the patients who responded favorably to drug therapy showed a marked increase in the actively proliferating population of bone marrow cells as early as 3 weeks after institution of therapy, when from the clinical consideration as well as from the morphological appearance of bone marrow it was not possible to predict that hematological remission would ensue several weeks later. In contrast, the group of patients in whom the therapy proved a failure either showed no increase or a marked decrease in the actively proliferating fraction of leukemic cells at 3 weeks after initiation of therapy, when, again, clinical and bone marrow appearances were as unhelpful prognosticating guides as they had been in the former group. This criterion of comparison of the fraction of proliferating pool of bone marrow cells, before and during therapy, may provide a useful tool for demonstration of the effectiveness of a therapeutic agent relatively early in the course of treatment of a patient. If the regimen is found unlikely to be effective, other therapeutic agents could be introduced before it becomes too late for that individual patient. In the studies reported in this paper, an exposure time of 6 days was used for autoradiography, but we have observed in subsequent work that this time can be reduced to 3 days. The *in vitro* LI of TdR-^{3}H can be measured within 4 days after the blood and bone marrow sample from a patient are obtained; therefore, this approach is practical in the clinical management of acute leukemia.

The mechanism of the increase in the proliferating fraction of bone marrow cells among the eventual responders to chemotherapy in our patients is not entirely clear, nor was the present investigation designed for the elucidation of that mechanism. One may also find this observation somewhat paradoxical because it apparently suggests that successful therapy increases the growth rate of that cell line (leukemic blasts) in which disappearance from the marrow and blood is the ultimate objective for remission induction. However, it may be possible to derive some explanation from the following: (*a*) as noted earlier (2, 12), the majority of the blast population of bone marrow in acute leukemia that is apparently nonproliferating is not an end-stage population, but maintains a capacity to reenter active cell cycle; and (*b*) it is likely that a feedback control exists (2, 4) in the kinetics of the cells in acute leukemia, as also indicated by Chan *et al.* (1), who observed an increased production of bone marrow cells following the destruction of leukemic blasts in the peripheral blood. The early (3 weeks after the institution of therapy, *i.e.*, much sooner than the onset of remission phase) marked increase in the proliferating fraction of the bone marrow cells among the responding group of patients may be due to a recruitment of the out-of-cycle leukemic blasts, which then become susceptible to the cytocidal effects of an agent lethal only to actively proliferating cells. During the stage of recruitment of cells into active cycle, the ratio of proliferating cells in the bone marrow would therefore be greater than during the stage of relapse and that prior to initiation of therapy (9).

The fraction of proliferating compartment in bone marrow among the responders was larger after 3 weeks of therapy than was the case eventually during remission (Table 2). At the latter time, the range as well as the mean LI were identical to those observed among the control bone marrows (Table 1). The increase in the LI at the time of remission is, however, not due to recruitment but to a return of the bone marrow to normalcy. Hart *et al.* have shown in their recent publications that (*a*) after 4 or 5 courses of chemotherapeutic agents in AML, the responding patients developed a marked increase in DNA synthesis incident to the proliferative thrust of normal bone marrow cellular elements (5), and (*b*) the patients with acute leukemia who had cytogenetic abnormalities lost the abnormality during complete remission, while at the time of relapse the abnormality recurred (6). Considered in this manner, by postulation of 2 separate explanations for the increase in the proliferating fraction of bone marrow cells, first during the early course of therapy and eventually during remission, the observations reported in this paper are no longer paradoxical. However, further work is required for objective evidence to support these postulates.

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