The Potentiality of Out-of-cycle Acute Leukemic Cells to Synthesize DNA

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The blood of 18 patients with acute leukemia was exposed to UV light. Within 2 hours, in all the patients (except one under chemotherapy), nearly 100 per cent of the leukemic blasts showed uptake of 3HTdR as a result of DNA repair replication. Similar exposures of CML blood white cells and chicken blood red cells to UV indicated that myeloid and erythroid cells have no capacity to repair DNA after they have stopped dividing as a result of maturation. This suggests that nonproliferating leukemia blasts are resting cells rather than "end cells."

It has been shown that leukemia of mice can be transmitted with a single cell. A theory of chemotherapeutic cure has developed from studies with the mouse L1210 leukemia in which it has been shown that almost all cells have the capability of dividing indefinitely and that a chemotherapeutic cure requires elimination of all the L1210 leukemic cells. This transplatable murine leukemia has been considered as a model for evaluation of agents to treat human leukemia. It is assumed in this model that human and murine leukemia are comparable. In contrast with L1210 leukemia, it is shown that there is a relatively large fraction of human leukemic cells that are apparently nonproliferating. Recently it has been shown that at least some of these apparent nonproliferating cells may reenter the cell cycle. The purpose of the present study is to determine the fraction of apparently nonproliferating cells which have retained the potentiality to synthesize DNA.

Materials and Methods

Eighteen patients with acute leukemia were studied. Eleven patients had acute myeloblastic leukemia: three boys 9 months, 19 months and 8 years old (respectively), and eight adults (five males and three females) whose ages ranged between 25 and 73 years. Seven patients had acute lymphoblastic leukemia: six children (two girls and four boys) whose ages ranged between 10 months and 9 years (one with mongolism—patient 6) and one 66-year-old man (Table 1). One 40-year-old female patient with chronic myeloid leukemia (CML) was also included in the study when her blood contained 150,000 white blood cells/cu. mm. with 20 per cent myelocytes and 2.5 per cent promyelocytes. Three
Acute lymphoblastic leukemia
1. D.P. M 6 y 100 Predn., MTX (-10 d)
2. F.S. M 9 y 47 70 Dauno., VCR, 6 MP (-3 d)
3. O.I. M 10 m 98 None
4. S.C. F 8 y 97 100 None
5. A.X. M 4 y 95 None
6. D.E. M 7 y 100 100 None
7. L.U. M 66 y 100 100 Predn., VCR.

Acute myeloblastic leukemia
8. A.V. M 9 m 100 100 None
9. B.O. M 19 m 94 None
10. G.M. M 8 y 100 100 None
11. G.J. M 30 y 95 100 None
12. V.R. F 30 y 100 100 None
13. A.A. M 48 y 95 100 Predn., CTX.
14. C.F. F 70 y 95 100 None
15. E.J. M 60 y 95 100 None
16. L.L. M 25 y 100 100 Predn., 6 MP (-3 d)
17. P.E. M 73 y 95 100 None
18. P.M. F 35 y 100 100 None

Predn., Prednisone; Dauno., Daunomycin; CTX, Cyclophosphamide; MTX, Methotrexate; VCR, Vincristine. (-3 d), the treatment was stopped 3 days before the test was performed.

patients (1, 2, 16) had received antileukemic treatment prior to the test, two patients (7 and 13) were under chemotherapy at the time of the study.

Nine ml. of venous blood were collected in a syringe containing 1 ml. of Dextran and 500 U. heparin. Red blood cells were allowed to sediment at 37°C for 30-60 minutes. Three ml. of the white cell rich supernatant were mixed with 10 ml. of tissue culture medium TC 199. This mixture was divided in 3-ml. samples which were spread in a sterile glass Petri dish in order to obtain a thin layer of approximately 0.5 mm. thickness. One sample was exposed for 30 seconds under a germicidal UV source which delivered the bulk of its energy at a wave length of 2537 Å. The UV dose delivered at a distance of 40 cm. (11 ergs/sq. mm./second) was measured by means of a Latarjet UV dosimeter. The sample, during irradiation, was gently agitated in order to distribute the dose of UV homogeneously to all the cells. The control sample was processed in the same manner except that the cover of the Petri dish was not removed under the UV source. Immediately after sham or actual irradiation, 0.3 ml. of reconstituted calf serum containing 3H-thymidine (3HTdR (Amersham, SA 5 Ci./mM) was added to all of the samples in an amount that yielded a concentration of 7 µCi./ml. in the cell suspension. This mixture was then incubated for 2 hours in sterile plastic tubes at 37°C. The white cells were then washed once in TC 199, and the pellet of white cells obtained by centrifugation was used to make cell smears. Duplicate slides were treated with DNase (Worthington Biochemical) (1 mg./ml). for 1 hour at 37°C at room temperature at pH 7.2. After fixation in methanol, the slides were dipped in NTB2 Kodak liquid nuclear emulsion diluted 1:1 with distilled water, exposed in the dark at 4°C for 18 and 70 days. Twenty-four hours after developing, the autoradiographs were stained with May-Grunwald Giemsa. One hundred to two hundred cells of each morphologic type investigated were examined for labeling on each slide. The background was estimated on the control slides. The labeling index of the irradiated samples was calculated by subtracting the background using a method similar to that described by Stillström.8
POTENTIALITY OF LEUKEMIC CELLS TO SYNTHESIZE DNA

Fig. 1.—Patient 14 with AML. The distribution of the non-S circulating myeloblasts according to the number of silver grains over the cell; irradiated (+) or not irradiated (−).

Fig. 2.—Patient 3 with ALL. The distribution of the non-S circulating lymphoblasts according to the number of silver grains over the cell; irradiated (+) or not irradiated (−).

RESULTS

Incubation of the nonirradiated samples with ³²HTdR determines the faction of leukemic cells that are in DNA synthesis (S). The non-S cells are either out of cycle, in G₁ or G₂. The unirradiated S cells were intensely labeled with grain counts overlying the cells in excess of 100. In contrast, after UV irradiation, a large population of labeled cells appear with maximum grain counts rarely in excess of 50. Thus it is easy to distinguish the cells in normal S from those in which ³²HTdR incorporation into DNA was induced by UV irradiation. In Table 1 it is seen that the per cent of labeled non-S circulating leukemic blasts in the UV irradiated samples ranged from 94 to 100 per cent with one excep-
Fig. 3.—The distribution of PMN seen in blood taken from a patient with acute leukemia according to the number of their silver grains. The exception was a patient who had been treated with daunomycin and vincristine until 3 days prior to the experiment. Only 70 per cent of the non-S leukemic cells in this treated patient were stimulated to incorporate $^3$HTdR into DNA. Eighty-five per cent of non-S leukemic blasts in the spinal fluid of patient 1 during an episode of leukemic meningeal infiltration were stimulated to incorporate $^3$HTdR after UV irradiation.

In Fig. 1, histograms of the grain count distributions overlying non-S UV-irradiated granulocytes:

- A: unirradiated granulocytes
- B: UV-irradiated granulocytes
- C: unirradiated non-S blasts
- D: UV-irradiated non-S blasts

Fig. 4.—Patient 3. The number of grains per 100 cells. A and B: unirradiated and irradiated segmented granulocytes. C and D: unirradiated and irradiated non-S blasts.
irradiated blasts and nonirradiated blasts are shown for patient 14 with AML. The highest grain count over unirradiated non-S cells was 8. In contrast grain counts overlying UV irradiated cells ranged up to 34. In Fig. 2, a similar histogram illustrates comparable differences between nonirradiated and irradiated cells from a patient with ALL.

In Fig. 3, the histograms of grain count distribution overlying UV-irradiated and nonirradiated granulocytes in blood of patient 3 with ALL is presented. The granulocytes were counted on the same slide as used for Fig. 2. Figure 4 compares the radioactivity, expressed as the number of grains per hundred (labeled and unlabeled) cells of the following types: (A) unirradiated PMN, (B) irradiated PMN, (C) unirradiated non-S blasts and (D) irradiated non-S blasts. Significant incorporation of ³HTdR was seen only in the UV-irradiated non-S blasts. Figure 5 compares the radioactivity expressed as the number of grains per hundred cells of each type of cell seen in UV-irradiated peripheral blood of a patient with CML. Non-S myelocytes (M₃-M₄) and lymphocytes (L) showed a similar uptake of ³HTdR which was of the same order of magnitude as seen in irradiated non-S leukemic blasts; metamyelocytes (M₅) showed a much lower uptake, whereas PMN (M₇) showed only a minimal uptake of ³HTdR above the background (C). The slides treated with DNase showed no labeling.

**DISCUSSION**

In human acute leukemia, several groups have reported the existence of a fraction of apparently nonproliferating cells among the blast cell population. At the time of diagnosis this fraction may represent 65–85 per cent⁴,⁵ of the bone marrow population. Whether all these leukemic blasts are only temporarily...
out of cycle, or whether a fraction of them are “end cells” which have lost forever the capacity to divide and are therefore destined to die, is the subject of the present report. Cabutti et al.\textsuperscript{6} have shown that following a sudden reduction of the leukemic blast population, in one patient with ALL, by treatment with methotrexate, the surviving blasts entered DNA synthesis. Mauer and Fisher,\textsuperscript{12} in one case of untreated ALL, presented evidence strongly indicating that the nonproliferating blasts may reenter the proliferating pool of blasts. These two observations indicate that at least some leukemic blasts are only temporarily out of cycle.

A large fraction of the small blood lymphocyte is a typical example of a non-dividing “dormant” cell capable of being stimulated to proliferate by mitogens or antigens under proper conditions. Unstimulated normal blood lymphocytes were shown by others\textsuperscript{9} to incorporate \(^{3}\)HTdR into DNA within 2 hours after a short exposure to UV light. This \(^{3}\)HTdR uptake following UV irradiation was shown to represent DNA repair replication.

The blood of one case of CML was studied by UV irradiation because it contained a mixture of actively dividing (myelocytes), nondividing (metamyelocytes and segmented neutrophils) and potentially dividing cells (lymphocytes) and therefore permitted a comparison of the response to UV in these different cell types. The myelocytes and blood lymphocytes showed a similar capacity to incorporate \(^{3}\)HTdR after UV irradiation. The \(^{3}\)HTdR uptake was of the same order of magnitude as seen in circulating leukemic blasts. Metamyelocytes are cells which during the process of maturation have lost the capacity to divide. Therefore they are never labeled in vivo after a pulse of \(^{3}\)HTdR. However, they showed a very low \(^{3}\)HTdR uptake after UV irradiation. Granulocytes, nondividing cells with an average life expectancy of a few hours, did not show significant \(^{3}\)HTdR uptake after UV irradiation. It has been shown by others\textsuperscript{10} that the capacity to initiate repair replication after exposure to UV light is high in proliferating myeloid cells and decreases progressively with maturation and the loss of the capacity to divide.

The UV test for repair replication capability in patients with acute leukemia was performed on peripheral blood, rather than on bone marrow, because it is known that the \(^{3}\)HTdR flash labeling index is lower in the blood than in the bone marrow in most cases of acute leukemia.\textsuperscript{11-13} Accordingly, it was felt the probability of finding blasts of the end cell type in the blood would be greater than finding them in the bone marrow. The uptake of \(^{3}\)HTdR by the irradiated cells represents incorporation into DNA since the radioactivity was removed by DNAase. This incorporation of \(^{3}\)HTdR into DNA probably represents DNA repair replication. In the myeloid series, cells lose the capacity to divide and initiate repair replication of DNA almost simultaneously. Similar unpublished observations have been made on the erythroid series. Some of the peripheral blood smears of acute leukemia contained a few orthochromatic erythroblasts which did not show incorporation of \(^{3}\)HTdR into DNA after UV irradiation, whereas all basophilic erythroblasts showed evidence for DNA repair. Moreover, in a study in progress, covering a variety of cell systems, it was found that nucleated circulating chicken erythrocytes do not enter DNA synthesis and do
POTENTIALITY OF LEUKEMIC CELLS TO SYNTHESIZE DNA

not show either evidence for DNA repair, whereas circulating chicken lymphocytes show evidence for DNA repair.

The observation that nonproliferating acute leukemic blasts after UV irradiation behave as lymphocytes and not as segmented granulocytes suggests that, like resting blood lymphocytes, the biochemical machinery necessary to resume cell replication is only temporarily repressed. These observations strongly suggest that nearly 100 per cent of non-S blasts are capable of reentering cycle since nearly 100 per cent are capable of DNA repair replication, showing they still retain DNA polymerase activity.

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REFERENCES

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