The Response of Chronic Lymphocytic Leukemia to Treatment by Extracorporeal Irradiation of the Blood, Assessed by Isotope-labeling Procedures


CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) is a clinical entity comprising a number of related disorders, the diversity of which is revealed both by the different immunologic and cytochemical properties of the lymphocytes concerned,1-4 and by the variety of behavior patterns displayed—notably in regard to progression of the disease and response to therapy. This diversity is also reflected in the response shown by patients with CLL to treatment by extracorporeal irradiation of the blood (ECIB).

Patients so far treated by this technic at the Royal Marsden Hospital include nine with various forms of lymphoma. Preliminary reports on the hematologic response and reduction in lymph node size achieved,5 as well as on studies of lymphocyte kinetics based on the analysis of radiation-induced chromosomal aberrations,6 have been published elsewhere.

In the past few years several investigators have reported their experience with this form of treatment and although the numbers of patients treated so far are small, three distinctive patterns of response are beginning to emerge: (1) a rapid fall in the peripheral white blood cell count during treatment, the count remaining low for a prolonged period afterwards2,3,7-12; (2) a less rapid fall in the white blood cell count during the course of treatment followed by a rapid return to pretreatment values2,12,13; (3) a slow or negligible fall in the peripheral white blood cell count during the course of treatment, the count, however, continuing to fall for a variable period after treatment has stopped and remaining at a low level for a time before rising again.13

It has been pointed out14 that information on the rate of exchange of leukocytes in the blood with those in the tissues, and on their distribution between these sites, is of "paramount importance" in treating leukemia by ECIB. This information can be derived from studies involving in vitro labeling and reinfusion procedures,14,15 which not only reveal the initial pattern of lymphocyte distribution but which, as will be shown below, can provide a quantitative...
assessment of response when repeated at the end of a course of treatment. To obtain an insight into the factors that operate to produce the different patterns of response observed, we have carried out such isotopic labeling studies in patients with CLL undergoing treatment by ECIB.

In this paper we discuss the results of such studies in three patients, each of whom exhibited one of the three characteristic patterns of response. All three presented with high peripheral leukocyte counts and gross splenomegaly. Two had undergone various courses of chemotherapy before being treated by ECIB, but one had received no previous treatment.

MATERIALS AND METHODS

Labeling Procedure

Leukocytes were labeled in vitro by a technic developed from that described by Fliedner.15 Approximately 500 ml. of blood were drawn into a sterile plastic bag containing 50 ml. of 1.5 per cent EDTA in 0.7 per cent NaCl. The bag was centrifuged at 10°C for 20 min. at 1000 × g. The plasma was transferred, by means of a "closed system" of plastic tubes into a second bag and theuffy coat layer, comprising about 50 ml., into a third bag. 250 μCi of tritiated uridine or cytidine (15-25 Ci./mM.) were added to the Buffy coat cells, which were then incubated for 45 min. at 37°C. The blood was then reconstituted by combining the contents of all three bags, without washing the Buffy coat cells of unincorporated isotope, and reinfused intravenously in 30-60 minutes.

Radiochemical Technics

A sample of the reconstituted blood was retained for analysis and 10-ml samples of peripheral blood were obtained at approximately daily intervals after infusion. Duplicate 3-4-ml aliquots of blood were added to 50 times their volume of 1 per cent acetic acid and after one hour at room temperature the precipitated nucleic acids were centrifuged and washed twice with water. The precipitates were thoroughly dried and were then combusted in oxygen in sealed flasks. The resulting tritiated water was taken up in 15 ml. of a liquid scintillation mixture consisting of naphthalene 200 Gm./L., PPO 7.0 Gm./L. and POPOP 0.3 Gm./L. in dioxane. Radioactivity was determined in a Tricarb β spectrometer, with a counting efficiency of 17-22 per cent: duplicate determinations agreed to within ± 5 per cent. Total nucleic acids were determined on 1-ml aliquots of blood by treating with acetic acid as before and extracting the precipitates with 1N perchloric acid at 80°C for 30 minutes; the optical densities of the extracts were measured at 260 μm.

Pool Size Calculation

The total exchangeable leukocyte pool was calculated from the degree of isotopic dilution of the labeled nucleic acids infused. A factor, previously determined, relating optical density at 260 μm. with hemocytometer count was used to derive cell numbers from nucleic acid values. The specific activity of nucleic acids in the exchangeable pool immediately after reinfusion was derived by plotting on semilogarithmic paper the corresponding specific activities in the blood found on subsequent days and extrapolating the best fitting straight line to "zero time."

A possible source of error with this technic could stem from the instability of the RNA labeling. There is evidence from autoradiographic studies15 that a substantial proportion of the label may be lost from circulating leukocytes within the first few hours of infusion. In deriving pool sizes in the present studies the specific activities of freshly labeled leukocytes before infusion and of circulating leukocytes at intervals after infusion, are compared. This comparison is probably valid since our preliminary experiments (unpublished) revealed that

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treatment with one per cent acetic acid (see Radiochemical Procedure, above) results in the elution of approximately the same amount of label from cells as is eluted in vivo. This loss is almost certainly due to label which is either incompletely incorporated into RNA or which is incorporated into short-lived—perhaps messenger—RNA. Moreover, values for relative sizes of extravascular and of circulating exchangeable pools of leukocytes, derived by our method have been in the same range as those deduced by others from autoradiographic data. However, although there may be some uncertainty in estimates of absolute pool size, the acetic acid precipitation-scintillation counting method should give valid estimates of relative change in pool size induced by treatment.

Irradiation

The extracorporeal irradiator consisted of a 440 Ci. 137Cs source encased in lead of minimum thickness 12.5 cm. Blood flowed through a coil of nylon tubing of 2.5-mm. internal diameter; the radiation dose-rate to the coil, determined by the ferrous sulphate method, was 5000 rad./min., the volume irradiated being about 5 ml. The flow rate was usually of the order of 70 ml./min., giving a transit dose of about 350 rad. The irradiation coil was coupled to a Scribner shunt inserted into a radial artery and a convenient vein in the forearm. A priming dose of approximately 7500 units of heparin was given intravenously at the start, and during treatment 10,000 units in 500 ml. of 4.3 per cent dextrose—0.18 per cent NaCl were infused through a T-connection into the arterial limb of the shunt at a rate sufficient to maintain a clotting time of 20–30 minutes.

Case Histories

Case 1. WS (Figs. 1 and 2). This 63-year-old man presented in February 1967 with an eight-month history of enlarged neck nodes. He was found on examination to have generalized lymphadenopathy and hepatosplenomegaly. The white blood cell count was 332,000/ cu.mm. with 99 per cent lymphocytes. More than 90 per cent of the bone marrow cells were
lymphocytes. Biopsy of a left axillary lymph node showed complete replacement of lymphoid tissue by well-differentiated lymphocytic cells. A lymphogram showed that pelvic and para-aortic nodes were enlarged with the typical abnormal lacy pattern seen in malignant lymphoma.

ECIB was given in six courses each of eight hours from February 22–28. The total white blood cell count fell to about 20,000 cells/cu.mm. during the period of treatment. There was marked regression of lymph nodes on both sides of the neck and in the left axilla, but there was no evidence of diminution in size of the spleen or of opacified abdominal nodes. The blood uric acid excretion rose from 0.94 Gm./24 hours on February 20 to 2.1 Gm./24 hours on February 23, falling to 0.77 Gm. on February 28.

The patient subsequently received radiotherapy to lymph node areas above and below the diaphragm, and was started on prednisone. His white blood cell count rose slowly and he died in December 1969, nearly two years after the ECIB.

Case 2: H1 (Figs. 3 and 4). This 63-year-old woman presented in April 1968 with a three-week history of joint pain and bruising. On examination she was found to have widespread indurated erythematous papules, some of which were purpuric; the spleen and liver were enlarged. The white blood cell count was 147,000/cu.mm. with 92.5 per cent lymphocytes, and 99 per cent of bone marrow cells were small lymphocytes. No peripheral nodes were palpable, and the chest radiograph and lymphogram were normal. She was started on prednisone, 60 mg./day.

ECIB was given in six courses each of seven-eight hours from May 17–23. This resulted in a fall in the total white cell count, which, however, rose rapidly on termination of treatment. The size of the liver and spleen remained unchanged. Despite attempts to control her disease with platelet transfusions, prednisone 100 mg. daily, vincristine and finally cyclophosphamide, she died on June 28, 1968.

Case 3: EK (Figs. 5 and 6). This 72-year-old woman presented in August 1964 with a three-month history of tiredness, weight loss and angina of effort. She was found to have an enlarged liver and spleen. The white cell count was 250,000/cu.mm. with 93 per cent

Fig. 2.—Chart showing percentage of radioactivity present in blood at different times following reinfusion of 3H-uridine labeled leukocytes, in patient W.S. (Case 1).
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Fig. 3.—Chart summarizing treatment history of patient H.V. (Case 2).

lymphocytes. She was started on prednisone and was treated at various times with chlorambucil, melphelan and cyclophosphamide without any marked change in the white cell count or reduction in the hepatosplenomegaly. In December 1964 radiotherapy was given to the enlarged spleen which was causing discomfort. A slow fall in the white cell count occurred in the following seven months, but by August 1967, despite treatment with cyclophosphamide, the count had risen to more than 200,000/cu.mm.

ECIB was started on September 13, 1967: 10 daily treatments each of eight hours followed by one five hour period two days later were given. There was no immediate response, but over the following three months the white cell count fell to 9500/cu.mm. and there was an associated slow rise in the hemoglobin concentration. There was progressive reduction in spleen size and the patient's general health improved markedly, her weight increasing by 14 lbs. The count subsequently rose slowly, reaching pretreatment levels about 10 months after ECIB. The rise in the white count was accompanied by an increase in spleen size.

One year after the first course of ECIB she received a second course, 12 treatments each of eight hours being given between September 13–27. The sequence of events which followed was almost identical to that observed after the first course. The minimum white count occurred five months after irradiation and was associated, as before, with a rise in the hemoglobin concentration and diminution in spleen size.

LABELING STUDIES: RESULTS AND DISCUSSION

Studies on patients with CLL treated by ECIB have revealed that extravascular lymphocyte pools may exhibit either rapid or relatively slow cellular exchange with the blood. The efficacy of treatment by ECIB hinges on the concept of fractional rate of destruction; that is, the proportion of the total
Fig. 4.—Specific activity of leukocytes (expressed as per cent of infused RNA-bound \(^3\)H-cytidine per 10\(^8\) cells) following reinfusion of in vitro labeled cells in patient H.V. (Case 2). Note constant rate of fall of specific activity, during and after ECIB and after repeating labeling procedure (indicated by arrow), despite rapid rise in peripheral leukocyte count following treatment.

The exchangeable pool destroyed per unit time. This rate may alter throughout treatment: it will increase if homeostatic mechanisms operate to maintain a high blood count by mobilizing cells from tissue depots; it will diminish with time if there is a tendency for lymphocytes to accumulate predominantly in extravascular tissues, as in some forms of lymphosarcoma. These principles are well illustrated by the three cases reported here (see Table 1).

In Case 1, labeling studies (Fig. 2) showed that approximately the same numbers of leukocytes were present in the blood as in tissue pools and that the rate of exchange was very rapid, equilibrium having been reached within one hour of reinfusing the labeled cells. Nearly one blood volume, equivalent to half the total exchangeable pool, therefore passed through the irradiation field every hour. Assuming that the leukemic lymphocytes exhibited radiosensitivities usual for mammalian cells and allowing for the random nature of the passage of cells through the irradiation field, it can be estimated that at least 30 per cent of cells in the total exchangeable pool were killed every hour.
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Fig. 5.—Chart summarizing treatment history of patient E.K. (Case 3).

Fig. 6.—Specific activity of leukocytes following in vitro labeling and reinfusion in patient E.K. (Case 3). Note accelerated fall in specific activity during ECIB. Symbols and ordinates as in Fig. 4.

This represents a very high fractional rate of destruction, and if this rate were maintained throughout the 48 hours of treatment, the leukocyte population would have been reduced by a factor of $3 \times 10^5$. In practice, however, the blood count was only reduced to 20,000 cells/cu. mm.: 1/22 of its pretreatment value. Evidently the fractional rate of destruction diminished sharply as treatment proceeded, implying a failure to mobilize the tissue stores of leukocytes. This conclusion was supported by the clinical observation that splenomegaly persisted despite the dramatic hematologic improvement.

In Case 2, labeling studies showed that lymphocytes in the peripheral cir-
Table 1.—Results of Isotopic Labeling Studies in Three Patients with Chronic Lymphocytic Leukemia

<table>
<thead>
<tr>
<th>Case Number</th>
<th>Blood Lymphocytes/cu.mm.</th>
<th>Per Cent of Labeled Cells Remaining in the Blood on Reinfusion</th>
<th>Total Pool of Dilution for Labeled Cells *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (W.S.)</td>
<td>449,000 a</td>
<td>ca. 50</td>
<td>$4.5 \times 10^{12}$</td>
</tr>
<tr>
<td>2. (H.V.)</td>
<td>110,000 a</td>
<td>9.6</td>
<td>$4.8 \times 10^{12}$</td>
</tr>
<tr>
<td>3. (E.K.)—1967</td>
<td>57,000 b</td>
<td>13.2</td>
<td>$2.2 \times 10^{12}$</td>
</tr>
<tr>
<td>1968</td>
<td>180,000 a</td>
<td>3.7</td>
<td>$14.0 \times 10^{12}$</td>
</tr>
<tr>
<td></td>
<td>259,000 a</td>
<td>3.5</td>
<td>$12.7 \times 10^{12}$</td>
</tr>
<tr>
<td></td>
<td>156,000 b</td>
<td>19.0</td>
<td>$4.0 \times 10^{11}$</td>
</tr>
</tbody>
</table>

a, b: At start and at end of ECIB, respectively.

* The derivation is described in the text.

culation initially represented about 1/10 of the total exchangeable pool. At the end of 50 hours of ECIB this ratio rose slightly although the total pool size was reduced to less than a half (Table 1). In contrast, therefore, to the response seen in Case 1, there was no failure to mobilize the tissue leukocytes, the tissue stores being reduced in step with the circulating pool. However, the rate of mobilization was comparatively slow, thus the count was only restored to its previous high value a week after the end of treatment.

Had this rise in leukocyte count after treatment been due to an accelerated rate of proliferation of leukemic cells, an increased rate of fall in their specific activity would have been seen. However, no change in the rate of fall of the specific activity, either during ECIB or following the course of treatment, or after relabeling of the cells was observed (see Fig. 4).

In this type of case, which superficially appears to be resistant to ECIB, the exchangeable lymphocyte pool is actually amenable to a considerably greater degree of depletion than in the type illustrated by Case 1. The prerequisite is to allow the peripheral count to rise between treatment periods and in this way to avoid a progressively diminishing fractional rate of destruction.

In Case 3, the mobilization of one class of cells from tissue stores was more evident. Autoradiography revealed that there were two classes of lymphocytes in the blood, one consisting of small cells which did not appear to incorporate tritiated uridine or cytidine, the other consisting of larger cells which incorporated label actively. Of the exchangeable pool of cells amenable to labeling, 3.5–3.7 per cent were present in the blood before ECIB; after 94 hours of treatment (second course) this proportion rose to 19 per cent (Table 1). There was thus a progressive rise in the fractional rate of destruction which resulted in a very extensive depletion of this population, from $12.7 \times 10^{12}$ cells initially to $4.0 \times 10^{11}$, i.e., to about three per cent. The rapid rate of destruction of this population was confirmed by the accelerated fall in radioactivity in the blood, observed to take place during both courses of treatment (Fig. 6).

In contrast, very little response was observed during the period of ECIB on the part of the nonlabeling population, which comprised the major fraction of the circulating leukocytes. Two explanations are possible: the cells could have been exceptionally radio-resistant if they were nondividing (as suggested
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by their deficient labeling) and did not suffer the interphase type of death characteristic for normal lymphocytes; alternatively, the fractional rate of destruction could have been low because extravascular pools were very extensive. It is tempting to conclude that these represented a long-lived progeny of the proliferating, readily-labeled population. Through the destruction of these precursors, the pool of nonproliferating cells ceased to be replenished, and in its turn became depleted as cells reached the end of their "natural" lifespan. This would account for the delayed profound reduction in the peripheral count, and in spleen size, observed after both courses of ECIB. If the blood picture reflected the cellular kinetics of the pool as a whole, the nonproliferating CLL lymphocytes had a halflife of about 12 days.

These three cases serve to emphasize the heterogeneous nature of this group of "accumulative" diseases collectively termed CLL. In different patients different mechanisms operate to regulate the distribution of leukocytes between the blood and tissue depots, as well as the degree and rate of cellular exchange between these sites. In some patients, exemplified by our Case 1, the blood may contain as many as half of the total readily exchangeable leukocytes in the body, but the tissue stores may not be easily mobilized; in others, as in our Cases 2 and 3, the cells in the blood represent a small fraction of the total, but tissue stores may be extensively—albeit sometimes slowly—mobilized to replace the cells destroyed in the blood.

Conclusions

ECIB has proved a useful form of treatment for certain types of CLL and has sometimes been the only effective treatment available (e.g., our Case 3). Hitherto, the choice of radiation dose rate, treatment schedule and duration has been empirical and guided primarily by the immediate response of the peripheral leukocyte count. To make the most effective use of this new therapeutic approach, however, requires a precise foreknowledge of the kinetic factors involved and a more rational assessment of response. Isotopic labeling studies can play a vital part in providing the information needed. For this purpose scintillation counting procedures are mandatory: autoradiography, owing to the time lag involved, would be impracticable.

Summary

The changes in circulating and extravascular lymphocyte pools that followed treatment by extracorporeal irradiation of the blood (ECIB) have been studied by isotopic labeling methods in three patients with chronic lymphocytic leukemia (CLL), each of whom exhibited one of three characteristic patterns of response. The patterns of response, and their interpretations in terms of cell kinetics, were as follows:

A rapid, sustained reduction in circulating leukocytes which occurs when extravascular pools are small in relation to the circulating pool.

A rapid rise in peripheral count after ECIB due to a transfer of cells from the extravascular depots when extravascular pools are relatively large and cellular exchange is slow.

A continuing fall in leukocyte count after ECIB resulting from a dif-
ferential depletion of proliferating cells when the circulating leukocytes consist both of nonproliferating end cells and of their precursors.

These studies have shown that in planning a treatment schedule it is important to determine the nature of the kinetic processes involved.

REFERENCES


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