Studies of Leukocyte Kinetics in Chronic Lymphocytic Leukemia

By Theodore S. Zimmerman, Herman A. Godwin, and Seymour Perry

SEVERAL STUDIES in normal animals and man have established that the bulk of circulating lymphocytes is non-proliferating and long-lived. Although a minority of cells have a short lifespan, most live for months or possibly years recirculating from the blood to the thoracic duct lymph passing through the lymph nodes, Peyer's patches and spleen. Utilizing a variety of lymphocyte labels, several investigators have shown that the predominant lymphocyte in chronic lymphocytic leukemia (CLL) is also long-lived with little proliferative potential. Recently it has been suggested that CLL is a disease in which these long-lived recirculating lymphocytes gradually accumulate resulting in enlargement of the lymphoid organs. Pooling of lymphocytes in the spleen appears to occur normally and would be expected in view of its role in recirculation of the lymphocyte. Studies with autologous chromium-51 labeled lymphocytes in a patient with CLL suggest that splenic pooling with subsequent release of lymphocytes occurs in this disease.

We have used radioautography and liquid scintillation counting to study the appearance of radioactivity in peripheral blood leukocytes of eleven patients with CLL after intravenous administration of the DNA pulse label, tritiated thymidine (H\textsuperscript{3}TdR). Our data indicate that in CLL, as in the normal individual, the long-lived lymphocyte predominates, but there is also a small population of more rapidly proliferating lymphocytes present. However, with progression of the disease, the long-lived lymphocyte accumulates in various organs.
Table 1.—Clinical Data: Patients With CLL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Race</th>
<th>Symptoms</th>
<th>Time Since Diagnosis (Years)</th>
<th>Symptoms</th>
<th>Adenopathy</th>
<th>Spleen Size (cm)</th>
<th>Liver Size (cm)</th>
<th>Hgb (gm%)</th>
<th>N* (per cu mm) (%)</th>
<th>Lt (%)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>F</td>
<td>W</td>
<td>0 Slight</td>
<td>2.5</td>
<td>0</td>
<td>Slight</td>
<td>0</td>
<td>0</td>
<td>11.9</td>
<td>95,400</td>
<td>6</td>
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<td>2</td>
<td>62</td>
<td>F</td>
<td>W</td>
<td>Adenopathy Marked</td>
<td>0.25</td>
<td>0</td>
<td>Marked</td>
<td>0</td>
<td>0</td>
<td>13.4</td>
<td>66,100</td>
<td>6</td>
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<tr>
<td>3</td>
<td>71</td>
<td>M</td>
<td>W</td>
<td>Weakness Slight</td>
<td>3.5</td>
<td>0</td>
<td>Slight</td>
<td>0</td>
<td>0</td>
<td>6.5</td>
<td>68,900</td>
<td>1</td>
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<tr>
<td>4</td>
<td>66</td>
<td>M</td>
<td>W</td>
<td>Fatigue, sweats, weight loss</td>
<td>3</td>
<td>0</td>
<td>Slight</td>
<td>0</td>
<td>0</td>
<td>7.2</td>
<td>114,000</td>
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<tr>
<td>5</td>
<td>60</td>
<td>F</td>
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<td>0 Slight</td>
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<td>0</td>
<td>0</td>
<td>13.3</td>
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<td>6</td>
<td>50</td>
<td>M</td>
<td>N</td>
<td>Fatigue, weight loss</td>
<td>6</td>
<td>0</td>
<td>Marked</td>
<td>3</td>
<td>3</td>
<td>5.5</td>
<td>832,000</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>F</td>
<td>W</td>
<td>Fatigue Marked</td>
<td>7</td>
<td>0</td>
<td>Marked</td>
<td>10</td>
<td>5</td>
<td>8.6</td>
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<td>8</td>
<td>51</td>
<td>M</td>
<td>W</td>
<td>Easy bruising</td>
<td>5</td>
<td>0</td>
<td>Slight</td>
<td>5</td>
<td>0</td>
<td>13.4</td>
<td>136,000</td>
<td>0</td>
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<tr>
<td>9</td>
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<td>F</td>
<td>W</td>
<td>0 Moderate</td>
<td>8</td>
<td>0</td>
<td>Moderate</td>
<td>4</td>
<td>9.8</td>
<td>80,300</td>
<td>1</td>
<td>99</td>
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<td>58</td>
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<td>W</td>
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<td>Adenopathy Massive</td>
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<td>0</td>
<td>Slight</td>
<td>2</td>
<td>0</td>
<td>13.1</td>
<td>50,400</td>
<td>7</td>
</tr>
</tbody>
</table>

*Neutrophils  †Monocytes  ‡Platelets  ††Erythroid  ‡‡Lymphoid  
†Lymphocytes  ‡Eosinophils  **Myeloid  ‡‡Lymphoid
### Table 1.—(Continued)

<table>
<thead>
<tr>
<th>Hemogram P&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Bone Marrow M&lt;sup&gt;−&lt;/sup&gt;&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Previous Treatment</th>
<th>Comment</th>
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<tr>
<td>Mt (%) E1 (%) per cu mm</td>
<td>M** (%) E&lt;sup&gt;++&lt;/sup&gt; (%) L&lt;sup&gt;++&lt;/sup&gt; (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 198,000</td>
<td>Normocellular, megakaryocytes present</td>
<td>15 15 70</td>
<td>None</td>
</tr>
<tr>
<td>1 111,000</td>
<td>Hypercellular, megakaryocytes present</td>
<td>5 5 90</td>
<td>None</td>
</tr>
<tr>
<td>38,000</td>
<td>Hypercellular, decreased megakaryocytes</td>
<td>0 0 100</td>
<td>Chlorambucil, nodal irradiation</td>
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<td>44,000</td>
<td>Hypercellular, occasional megakaryocytes</td>
<td>5 5 90</td>
<td>Thio-tepa</td>
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<tr>
<td>1 115,000</td>
<td>Normo- to hypercellular, megakaryocytes</td>
<td>10 10 80</td>
<td>None</td>
</tr>
<tr>
<td>136,000</td>
<td>Hypercellular, megakaryocytes present</td>
<td>0 0 100</td>
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<td>207,000</td>
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<td>10 5 85</td>
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</tr>
<tr>
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<td>Normocellular, increased megakaryocytes</td>
<td>10 15 75</td>
<td>Chlorambucil, methylprednisolone</td>
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<tr>
<td>1 52,000</td>
<td>—</td>
<td>—</td>
<td>Splenectomy because of thrombocytopenia</td>
</tr>
<tr>
<td>232,000</td>
<td>Hypercellular, megakaryocytes present</td>
<td>1 1 98</td>
<td>Chlorambucil</td>
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<tr>
<td>—</td>
<td>Hypercellular, increased megakaryocytes</td>
<td>0 0 100</td>
<td>Chlorambucil Died of E. coli pneumonia</td>
</tr>
<tr>
<td>1 2 282,000</td>
<td>Normocellular, absent megakaryocytes</td>
<td>5 5 90</td>
<td>None</td>
</tr>
<tr>
<td>2 71,000</td>
<td>Hypocellular, megakaryocytes present</td>
<td>5 30 65</td>
<td>Total body irradiation Study performed after total body irradiation</td>
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</table>
sites throughout the body. One consequence of this accumulation appears to be increased splenic pooling, splenomegaly, and occasionally hypersplenism.

**Methods and Materials**

The diagnosis of CLL rested on the finding of otherwise unexplained persistent lymphocytosis. We studied 10 patients in relapse and one patient in partial remission (Table 1). The 10 patients in relapse, and the patient in partial remission prior to treatment, all had peripheral blood lymphocyte counts above 20,000 per cu. mm. and bone marrow specimens infiltrated with over 65 percent lymphocytes. We also studied a 48-year-old woman who had recently had a malignant melanoma removed from her left elbow. An axillary metastasis was present. She was hematologically normal as shown by bone marrow examination and determination of hemoglobin, peripheral white blood cell and differential counts and platelet count. All patients were beyond the reproductive age group.

Tritiated thymidine (S.A. 1.9 or 14 C per mmole),* 75 μc. per kg. body weight, was injected intravenously in a single dose. Aliquots (2-20 ml.) of peripheral blood were drawn just before the injection of the isotope and at predetermined intervals thereafter. Five percent EDTA was used as the anticoagulant. Leukocyte counts were done using an electronic cell counter.† Leukocytes were isolated by dextran sedimentation and hypotonic lysis as previously described.‡ Cell buttons containing 10⁸ cells were prepared by centrifugation at 2,100 g for 10 minutes. These were dissolved in 0.5-1.0 cc. of NCS® reagent and following the addition of 15 cc. of toluene phosphor scintillation fluid, radioactivity was determined in a liquid scintillation spectrometer.* Correction for quenching was by means of an external standard. Radioactivity was expressed as counts per minute per 10⁸ cells.

Smears of whole blood for radioautographs were made on clear gelatin-coated microscope slides, air dried and fixed in absolute methanol. Kodak AR-10 stripping film was applied, and the slides were exposed for 5–8 months at 4 C. The radioautographs were then developed, fixed, stained with Giemsa stain, and 200–5,000 cell counts were performed on each slide, depending on the proportion of labeled cells identified. Background was less than one grain per cell and therefore no correction was applied, but no cell was considered labeled which had less than three grains. Lymphocytes were classified into large and small according to accepted criteria.§ No radioautographs were available for analysis from patient Nos. 1, 6, and 10 because of technical difficulties.

**Results**

The peripheral blood leukocyte radioactivity curve following in vivo injection of H³TdR in a hematologically normal individual is shown in Figure 1 and is similar to previously reported studies.⁷ Note that the level of radioactivity remained quite low in the peripheral blood until approximately 120 hours after labeling. Peripheral leukocyte radioactivity then sharply increased, reaching peak levels at about 165 hours. By referring to the radioautographic analysis (Table 2), it can be seen that the early radioactivity was primarily due to labeled lymphocytes, but radioactivity giving rise to the major peak came largely from labeled neutrophils.

The peripheral blood leukocyte radioactivity curves in patients with CLL in relapse may be classified into two distinct patterns. Pattern No. 1 (present in 5 patients) had a relatively high peak of specific activity (Figure 2) occurring

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†Coulter Electronics, Hialeah, Florida.
between 80 and 192 hours after administration of H³-TdR. In these patients, analysis of radioautographs (Table 3) revealed that the early radioactivity was primarily due to labeled lymphocytes. The lymphocyte labeling index increased to a maximum in each patient in conjunction with peak leukocyte radioactivity. Initially no labeling was present in neutrophils, but these gradually began to appear so that at the time of peak leukocyte radioactivity, these constituted the predominant labeled cell. The labeling index of lymphocytes and neutrophils decreased in conjunction with the fall-off of leukocyte radioactivity as measured by liquid scintillation counting. Lymphocyte labeling was still detected at the end of the longest study (56 days). Patients demonstrating
Fig. 2.—Pattern No. 1. Leukocyte specific radioactivity curves from patients with C.L.L. in relapse.
Pattern No. 2. Leukocyte specific activity curves from patients with CLL in relapse.
pattern No. 1 were also distinguished by having generally lower peripheral lymphocyte counts and no detectable hepatosplenomegaly.

Pattern No. 2 consisted of a nearly flat leukocyte specific radioactivity curve with minor fluctuations without a distinct isolated peak of radioactivity (Figure 2). The low level of radioactivity generally observed in this pattern is similar to the terminal phase in pattern No. 1. Radioautographic analysis (Table 4) in pattern No. 2 showed a distinctly lower lymphocyte labeling index compared with the labeling index seen in the first group. Similarly, no peak of neutrophil labeling was observed and the neutrophil labeling index was usually much lower than in the first group of patients. There were no significant fluctuations.
in labeling during the period of study. Pattern No. 2 was seen only in patients with splenomegaly and, with one exception, hepatomegaly. These patients also tended to have higher lymphocyte counts than the first group. Patient
Fig. 4.—The effect of splenectomy on the leukocyte specific activity curve from a patient with CLL in relapse.
No. 7 is somewhat atypical in that a modest increase in leukocyte radioactivity was detected at 336 hours after administration of H\(^3\)TdR. However, radioautographic analysis revealed the low labeling index characteristic of this group (Table 5).

A variation in the leukocyte radioactivity patterns was seen in patient Nos. 2, 6, 7, and 8. During the first few hours following H\(^3\)TdR administration a rapid drop in leukocyte radioactivity occurred. Radioautographic analysis showed most of this early radioactivity to reside in large lymphocytes. The remaining portions of the leukocyte radioactivity curves from these patients were similar to those of the other patients.

One patient with pattern No. 2 was studied before and after splenectomy for refractory thrombocytopenia. The leukocyte radioactivity curve prior to treatment was flat (Figure 4) with a very low labeling index (Table 4). Only one labeled neutrophil was seen during the entire study. Following splenectomy, a peak appeared consisting of labeled lymphocytes and neutrophils. The labeling index of both cell types was significantly greater than in the first study (Table 4). The platelet count rose from 13,000 to 52,000 per cu. mm. and the percentage of neutrophils rose from 0.3 percent to 8 percent with no change in peripheral white blood count. Eight months following splenectomy, the WBC was still unchanged with 10 percent neutrophils and 90 percent lymphocytes and a platelet count of 81,000 per cu. mm.

Another patient (No. 11) with partial bone marrow and peripheral blood remission induced by fractionated total body radiation showed a curve similar to that seen in normal individuals. However, an atypical early gentle rise prior to the sharp increment in leukocyte radioactivity was seen. The lymphocyte labeling index was much higher than observed in the patients in complete relapse but not as high as in the hematologically normal individual (Tables 1 and 5).

**DISCUSSION**

Several studies have suggested that there is a short-lived as well as a long-lived population of lymphocytes in animals and man.\(^6,8\) The results of our studies are consistent with the presence of two such populations in CLL with an increasingly greater preponderance of longer-lived non-proliferating cells as organomegaly develops and the lymphocyte count rises. In patients with pattern No. 1, a small but definite influx of labeled lymphocytes into the peripheral blood is discernible in conjunction with the rise in leukocyte specific radioactivity although much of this radioactivity is due to labeled neutrophils. Most of these lymphocytes along with the labeled neutrophils leave the circulation during the phase of relatively rapid decrease in leukocyte radioactivity. However, some labeled lymphocytes continue to circulate during the more gradual decline of radioactivity observed in the latter part of the study. In pattern No. 2 there is a prolonged low level of radioactivity with no significant peak at any time. The decline in radioactivity is gradual as in the latter portion of pattern No. 1. It would appear that in those patients with pattern No. 1, the majority of newly formed lymphocytes spend a relatively brief time in the peripheral blood with only a small proportion of them remaining for a long
time. However, only a relatively small number of long-lived cells needs to be produced each day to result in a gradual accumulation.

In pattern No. 2, very few lymphocytes were labeled indicating that the population consisted almost entirely of older, long-lived non-proliferating cells. Significant organomegaly was present only in this group of patients suggesting that organ enlargement in CLL is the result of accumulation of these long-lived nondividing lymphocytes as has been proposed by others.16,17

Investigators have previously studied patients with CLL using a variety of in vivo lymphocyte labels. Cronkite et al.15 gave H3TdR to one patient and monitored the appearance of peripheral blood leukocyte radioactivity by radioautographic analysis. This patient seems to fall into our first pattern but sufficient data are not given to allow definitive classification. Hamilton,11 using C14-labeled DNA precursors, and Christensen and Ottesen,15 Osgood et al.12 and Perry et al.22 using P32 as DNA labels, obtained leukocyte specific radioactivity curves similar to those found in our first group. However, the type of cells labeled were not identified. It is of interest that the patient described in the paper by Christensen and Ottesen had no organomegaly as is characteristic of patients in the first group. The specific activity curves obtained by Weisberger and Levine13 with S35 cystine and those of Walker et al.23 using P32 fit our second category, but the physical findings were not described.

The therapeutic response of CLL is consistent with the hypothesis that the disease reflects an accumulation of recirculating, long-lived, nondividing lymphocytes. Complete remissions are rare and usually have been accomplished with radiotherapy.24-27 In one of our patients (No. 11), partial remission was achieved with fractionated total body irradiation. The specific activity curve obtained by liquid scintillation counting approached the normal pattern. However, analysis of the radioautographs revealed a higher lymphocyte labeling index compared to the patients in relapse although less than in the hematologically normal individual. This suggests that a preferential destruction of nonproliferating lymphocytic tissue was achieved by the radiotherapy. The primary effect of irradiation is the destruction of mature lymphocytes, but there may also be a “feedback” block in lymphocyte production.28-30 Extracorporeal and fractionated total body irradiation in treatment of CLL results in the destruction of large numbers of lymphocytes and is beneficial.27,31 In addition, alkylating agents, which are of proven efficacy in CLL are thought to cause interphase as well as mitotic death.32 Similarly, corticosteroids are toxic to mature lymphocytes although they can also block lymphopoiesis.33,34 Agents effective in CLL all seem to possess the ability to attack the mature long-lived, nondividing lymphocytes which apparently comprise the bulk of excess tissue in this disease.

There is considerable evidence indicating that there is a population of long-lived lymphocytes in normal individuals, in patients with CLL and in animals.3,6,11-14 In the present study, the data indicate that CLL lymphocytes may survive for at least 56 days (the duration of the longest study). However, the possibility of reutilization of label makes definitive interpretation of the data on this particular point difficult.
The concept of lymphocyte recirculation and pooling has generally been accepted. Gowans has demonstrated with radioisotopically labeled lymphocytes that the route of recirculation of lymphocytes in the rat from the bloodstream to the thoracic duct is through the lymph nodes, Peyer’s patches, and spleen. Thus, these organs represent a readily available pool of lymphocytes. Using the sex chromosome as a lymphocyte marker, Perry et al. were able to demonstrate that recirculation of lymphocytes from the blood to the thoracic duct occurs in man. The early rapid drop in leukocyte specific radioactivity noted in some of our patients (Nos. 2, and 6–8) probably represents pooling of cells in lymphoid tissue. Pooling of CLL lymphocytes in the spleen with subsequent slow release has been suggested by the results of Cr51-labeled lymphocyte studies in patient No. 10.

The influence of the spleen on the pattern of leukocyte radioactivity in the peripheral blood was demonstrated in patient No. 8, who was studied with H3TdR before and after splenectomy for refractory thrombocytopenia. Prior to splenectomy, the proliferating population of lymphocytes was barely detectable. After splenectomy, the leukocyte radioactivity pattern was characteristic of that group of patients without organomegaly, and a small population of newly produced cells was not clearly evident. It seems likely that the patient’s large spleen was a storehouse for long-lived, non-proliferating recirculating lymphocytes. Removal of this population of cells increased the relative proportion of young labeled cells. Alternatively the spleen may have suppressed lymphopoiesis through some humoral mechanism. Platelet and neutrophil counts increased markedly after splenectomy as did the neutrophil labeling index. The explanation for the latter finding is not clear but at least two possibilities may be considered. There may have been selective sequestration of newly formed neutrophils in the spleen or this organ could have exerted a suppressive effect on myelopoiesis.

**Summary**

Intravenously administered H3TdR was used to study peripheral blood leukocyte kinetics of ten patients with CLL in relapse and one in partial remission. Two distinct patterns were found. The first pattern indicates the presence of a small, proliferating population of lymphocytes which is present in the blood for a relatively brief time. This pattern was found in patients with no organomegaly and with relatively lower peripheral lymphocyte counts. In the second pattern almost all of the cells present were non-proliferating and long-lived. The patients with the latter pattern had organomegaly. These studies support the concept that there is a progressive widespread accumulation of long-lived lymphocytes in CLL often leading to splenic pooling and, in some cases, hypersplenism.

**ACKNOWLEDGMENT.**

The authors wish to express their appreciation to Dr. Ralph Johnson and Dr. Jerome Block of the National Cancer Institute for allowing us to study their patients and to Miss Ada Brooks for providing technical assistance.

Everett, N. B.: Radioautographic studies of lymphocyte longevity. Extreme accumulation de longev lymphocytos in chronic leucemia lymphocytic que resulta hhabeva organomegalia. Le studios supporta le conception que ii existe un progressive e frequentemente in un concentration in le splen e, in certe presentes esseva non-proliferante e de alte longevitate. Le patientes con iste configuration numerations peripheric de lymphocytos. In iste configuration esseva trovate in patientes sin organomegalia e con relativamente basse numerations peripheric de lymphocytos. Le first in un con le mesme condition in remission. Duo distincte configurationes esseva trovate. Ab sanguine peripheric in dece patientes con chronic leucemia lymphocytic in recidiva e isto es presente in le sanguine solo durante un relativamente breve periodo de tempore. Le presents supports the conception that it exists a progressive and extensive accumulation of long-lived lymphocytes in chronic lymphocytic leukemia that results frequently in an concentration in the spleen, in certain cases, in hypersplenism.

REFERENCES

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THEODORE S. ZIMMERMAN, HERMAN A. GODWIN and SEYMOUR PERRY