The Mechanism of the Development of Anemia in Untreated Chronic Lymphatic Leukemia

By PRAWASE WAsI* and MATLW BLOClk

INTRODUCTION

ANEMIA is part of the natural history of chronic lymphatic leukemia. The etiology of the anemia has been attributed to a decreased rate of red cell production1-3 and to an increased rate of red cell destruction.4-9 Prior studies have been based on estimations of (1) the amount of erythroblastic tissue found in smears of aspirated bone marrow and in sections of the marrow at autopsy; (2) the red cell survival by Ashby or radiochromium technics; and (3) the rate of red cell formation by radioiron technics and reticulocyte counts. Furthermore, investigators of this problem generally have not taken into account the effect of therapy on the development of anemia.

The purpose of this investigation was to study the interrelationships of the peripheral blood findings, the histopathology of the bone marrow, and the red blood cell survival in order to elucidate the mechanism by which anemia develops in patients with untreated chronic lymphatic leukemia.

MATERIALS AND METHODS

1. Clinical Material: The subjects were 20 consecutive patients with untreated chronic lymphatic leukemia. No patient with an autoimmune hemolytic disease was encountered in this series. The diagnosis was based on an absolute lymphocytosis (table 1) and bone marrow infiltration with small lymphocytes without other cause.

2. Histopathologic Study: Bone marrow specimens were obtained from the sternal marrow by aspiration with an 8-gauge needle. The tissue was fixed in neutral Zenker-formol solution (9:1 dilution) for 1-2 hours. It was then processed by a modified Maximow technic (sectioning in nitrocellulose at 4 µ or 6 µ and staining with hematoxylin eosin-azure II).10 The relative amount of fat, myeloid (erythroblastic, granulocytic, and megakaryocytic) and lymphatic tissue was estimated by examining sections of each specimen of sternal marrow as an unknown. The percentages listed in the table under "Bone Marrow," some of which are illustrated in figures 3-5, represent an over-all average of the entire marrow section. The areas of fat, lymphatic and myeloid tissue were estimated and not measured, and cell counts were not done.

3. Red Blood Cell Survival: The method used by Weinstein and Leroy8 was followed with only minor modifications. Ascorbic acid was not added to the mixture of radiochromium and blood after incubation since the blood volume was not determined. Correction for plasma radioactivity was not necessary after the first day since significant amounts of radioactivity were not demonstrable in the plasma after 24 hours.

The radioactivity of the blood samples expressed in counts per volume per minute was

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Fig. 1.—Red cell survival curves of 14 patients without anemia (Group I). The shaded area represents the normal range.

Fig. 2.—Red cell survival curves of 6 anemic patients (Group II). The shaded area represents the normal range.
plotted on ordinary graph paper against time in days. A straight line could usually be drawn through the points (figs. 1 and 2). The line was then extrapolated to zero time. The value at the point of interception with the ordinate was taken as 100 per cent activity. The time in days when 50 per cent of the radioactivity disappeared was called the apparent half life of the red blood cells.

RESULTS

1. Clinical and Peripheral Blood Findings

A summary of the clinical and laboratory data is given in table 1. The exact duration of the illness was not known because most of the patients were asymptomatic; in some patients the onset was dated from the time that the patient was first aware of lymphadenopathy. Three patients (cases 6, 19, 20) noted fatigue and weight loss. Case 6 had pain in the left upper quadrant probably from splenic infarction. Slight, localized lymphadenopathy was detected in most cases. In the majority of the patients the spleen varied from being just palpable to 15 cm. below the costal margin.

In 15 of the patients the disease was accidentally discovered when white blood counts were obtained as part of an examination unrelated to symptoms ascribable to leukemia. In two patients (cases 5 and 16) white counts were available prior to the estimated onset of the leukemia. The absolute number of lymphocytes in these two patients increased gradually during the seven years after the diagnosis of leukemia had been made.

The patients were divided into 2 groups on the basis of hemoglobin level. In Group I (cases 1–14) the hemoglobin varied from 14.0 to 17.8 Gm. per cent. In Group II (cases 15–20) the hemoglobin concentration ranged between 9.3 and 12.7 Gm. per cent.

2. Histopathology of the Bone Marrow

Myeloid tissue in the adult normally occupies from 20 per cent (rarely, in elderly individuals, only 15 per cent) to 40 per cent of the sternal marrow, the remainder being fat. The concentration of myeloid tissue gradually decreases with age. Erythroblasts form approximately 40 per cent of this myeloid tissue. One or two small lymphatic nodules composed of dense sheets of small lymphocytes, usually well demarcated from surrounding myeloid tissue and fat, are commonly observed in an average-size biopsy in individuals over 50 years of age.

The relative amount of myeloid, fat and lymphatic tissues in 20 patients with untreated chronic lymphatic leukemia is presented in table 1. In Group I an increased amount of lymphatic tissue composed of small foci of small lymphocytes was always found in the marrow (fig. 3). In addition, in some patients in Group I large nodules of lymphatic tissue were found (fig. 4). The range of amount of lymphatic tissue in Group I is listed in table 1. Representative areas are show in figures 3 and 4. Except for Case 9 the amount of myeloid

*In all patients 40 per cent of the myeloid tissue was composed of erythroblasts (figs. 3C, 4C and 5C).
Figs. 3, 4 and 5.—See legend, facing page.
tissue was in the normal range; any increase in lymphatic tissue was at the expense of the fat (figs. 3 and 4). The hemoglobin of Case 9 fell to 5.5 Gm. per cent in five months.*

In Group II there was a marked replacement of myeloid tissue and fat by lymphatic tissue so that the marrow consisted primarily of sheets of lymphocytes with only rare myeloid cells (fig. 5 and table 1). In some patients (Cases 18 and 19), the fat was almost replaced.

The amount of iron in the bone marrow of patients in both groups varies from a trace to 3+ (the amount seen in hemochromatosis being classified 4+ and in normal adult males as 1+).

3. Red Cell Survival (figs. 1 and 2 and table 1)

The apparent half survival time of the red cells by the Cr¹⁹⁵ technic in seven normal subjects (medical students and hospital staff) ranged from 29 to 36 days. Six of the seven normal subjects ranged from 29 to 32 days.

Eleven of the patients in Group I had an apparent red cell half life between 29 and 36 days. The other three patients in this group had an apparent red cell half life of 26, 26 and 28 days.

Five of Group II patients had an apparent red cell half life between 29 and 35 days. The sixth and most anemic patient in this group (Case 20) had an apparent red cell half life of 19 days. This patient died within one year of this study.†

4. Correlation of Data

A. Lymphocytosis and the concentration of marrow lymphatic tissue: The relation between the number of lymphocytes in the peripheral blood and the

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*The authors would like to thank Drs. John Elston and A. A. Lampert of Rapid City, S. D., for their cooperation in following this patient.
†The follow-up history and marrow smears were kindly supplied by Dr. Ernest Beutler.
Table 1.—A Summary of the Clinical and Laboratory Data in 20 Patients with Untreated Chronic Lymphatic Leukemia in Order of the Hemoglobin Level

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age</th>
<th>Sex</th>
<th>from symptoms</th>
<th>since diagnosis established</th>
<th>Splenomegaly</th>
<th>Lymphadenopathy</th>
<th>Hgb Gm.%</th>
<th>Lymphocytes per mm³</th>
<th>% Myeloid</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.S.</td>
<td>55</td>
<td>M</td>
<td>4-5 yrs.</td>
<td>2 wks.</td>
<td>3 cm.</td>
<td>+++</td>
<td>17.8</td>
<td>50,000</td>
<td>12</td>
</tr>
<tr>
<td>R.S.</td>
<td>63</td>
<td>M</td>
<td>—</td>
<td>20 mos.</td>
<td>—</td>
<td>+</td>
<td>16.6</td>
<td>23,000</td>
<td>24</td>
</tr>
<tr>
<td>J.C.</td>
<td>65</td>
<td>M</td>
<td>—</td>
<td>2 mos.</td>
<td>7 cm.</td>
<td>—</td>
<td>16.2</td>
<td>102,000</td>
<td>12</td>
</tr>
<tr>
<td>R.F.</td>
<td>68</td>
<td>M</td>
<td>—</td>
<td>2 yrs.</td>
<td>—</td>
<td>—</td>
<td>16.1</td>
<td>53,000</td>
<td>12</td>
</tr>
<tr>
<td>C.P.</td>
<td>86</td>
<td>F</td>
<td>—</td>
<td>7 yrs.</td>
<td>—</td>
<td>+</td>
<td>15.8</td>
<td>23,000</td>
<td>12</td>
</tr>
<tr>
<td>J.T.</td>
<td>67</td>
<td>M</td>
<td>3 yrs.</td>
<td>1 mo.</td>
<td>1.5 cm.</td>
<td>+</td>
<td>15.4</td>
<td>23,200</td>
<td>24</td>
</tr>
<tr>
<td>E.S.</td>
<td>51</td>
<td>M</td>
<td>5-6 yrs.</td>
<td>3 mos.</td>
<td>3 cm.</td>
<td>+++</td>
<td>15.3</td>
<td>36,200</td>
<td>12</td>
</tr>
<tr>
<td>M.K.</td>
<td>47</td>
<td>M</td>
<td>—</td>
<td>18 mos.</td>
<td>tip</td>
<td>+</td>
<td>15.0</td>
<td>24,800</td>
<td>Infiltrated with lymphocytes*</td>
</tr>
<tr>
<td>A.G.</td>
<td>69</td>
<td>M</td>
<td>—</td>
<td>2 mos.</td>
<td>2 cm.</td>
<td>+</td>
<td>15.0</td>
<td>21,700</td>
<td>6</td>
</tr>
<tr>
<td>E.F.</td>
<td>67</td>
<td>F</td>
<td>—</td>
<td>18 mos.</td>
<td>—</td>
<td>+</td>
<td>14.8</td>
<td>25,600</td>
<td>12</td>
</tr>
<tr>
<td>R.G.</td>
<td>65</td>
<td>F</td>
<td>—</td>
<td>2 mos.</td>
<td>3 cm.</td>
<td>—</td>
<td>14.7</td>
<td>20,800</td>
<td>9</td>
</tr>
<tr>
<td>H.P.</td>
<td>65</td>
<td>M</td>
<td>—</td>
<td>3 mos.</td>
<td>—</td>
<td>+</td>
<td>14.5</td>
<td>22,400</td>
<td>9</td>
</tr>
<tr>
<td>R.L.</td>
<td>67</td>
<td>F</td>
<td>—</td>
<td>1 mo.</td>
<td>—</td>
<td>—</td>
<td>14.5</td>
<td>11,400</td>
<td>12</td>
</tr>
<tr>
<td>H.F.</td>
<td>64</td>
<td>M</td>
<td>—</td>
<td>3 yrs.</td>
<td>1 cm.</td>
<td>+</td>
<td>14.0</td>
<td>58,500</td>
<td>15</td>
</tr>
<tr>
<td>E.H.</td>
<td>55</td>
<td>M</td>
<td>—</td>
<td>1 yr.</td>
<td>tip</td>
<td>+</td>
<td>12.7</td>
<td>103,000</td>
<td>Extensively infiltrated with lymphocytes*</td>
</tr>
<tr>
<td>E.H.</td>
<td>85</td>
<td>M</td>
<td>—</td>
<td>7 yrs.</td>
<td>3 cm.</td>
<td>+</td>
<td>12.2</td>
<td>115,000</td>
<td>6</td>
</tr>
<tr>
<td>R.B.</td>
<td>39</td>
<td>M</td>
<td>—</td>
<td>1 mo.</td>
<td>2 cm.</td>
<td>+++</td>
<td>12.0</td>
<td>398,000</td>
<td>6</td>
</tr>
<tr>
<td>E.S.</td>
<td>48</td>
<td>F</td>
<td>—</td>
<td>8 yrs.</td>
<td>1 cm.</td>
<td>+</td>
<td>10.6</td>
<td>101,000</td>
<td>6</td>
</tr>
<tr>
<td>J.W.</td>
<td>58</td>
<td>M</td>
<td>3-4 yrs.</td>
<td>1 mo.</td>
<td>6 cm.</td>
<td>+++</td>
<td>10.3</td>
<td>408,000</td>
<td>3</td>
</tr>
<tr>
<td>T.R.</td>
<td>46</td>
<td>M</td>
<td>2 mos.</td>
<td>1 wk.</td>
<td>15 cm.</td>
<td>+++</td>
<td>9.3</td>
<td>746,000</td>
<td>Almost 100% lymphatic tissue†</td>
</tr>
</tbody>
</table>

Note: + = mild localized lymphadenopathy, lymph nodes smaller than 1.5 cm. in diameter.
++ = localized lymphadenopathy, lymph nodes larger than 1.5 cm. in diameter.
+++ = mild generalized lymphadenopathy, lymph nodes smaller than 1.5 cm. in diameter.
++++ = generalized lymphadenopathy, lymph nodes larger than 1.5 cm. in diameter.

*Specimens were not good enough for an accurate estimation.
†Only smears available.
ANEMIA IN CHRONIC LYMPHATIC LEUKEMIA

relative amount of lymphatic tissue in the bone marrow in 25 patients with untreated chronic lymphatic leukemia is presented in figure 6 and table 1. There is a direct correlation between the number of lymphocytes and the concentration of marrow lymphatic tissue when at least 50 per cent of the marrow was replaced by lymphatic tissue.

B. Myeloid and lymphatic tissues: Figure 7 and table 1 show that in general the amount of myeloid tissue was less than normal only in those patients in whom the lymphatic tissue constituted more than 50 per cent of the marrow.

C. Hemoglobin level, myeloid tissue and red cell survival (figs. 3, 4, 5 and 8):

NONANEMIC PATIENTS (GROUP I): A normal amount of myeloid tissue was present in all patients in this group with the exception of Case 9 (figs. 3, 4 and 8). The variation in hemoglobin and in amount of myeloid tissue was similar to what is seen in normal adults. In Case 9, myeloid tissue constituted only 10 per cent of the marrow at a time when the hemoglobin was 15 Gm. per cent but as has already been mentioned, within six months the hemoglobin decreased to 5.5 Gm. per cent. The red cell survival was also normal in every patient except in Cases 5, 8 and 12 with apparent half cell survival respectively of 28, 26 and 26 days. These figures are only slightly below the range of normal in this laboratory.

ANEMIC PATIENTS (GROUP II): The amount of myeloid tissue was less than normal in this group (figs. 5 and 8). In addition, as shown in figure 8, a rough correlation was observed between the decrease in myeloid tissue and the degree of anemia. The apparent red cell half life was within the normal range in all patients of this group with the exception of Case 20.

An interesting observation was that patients in this group consistently had lymphocyte counts over 100,000 whereas only one patient in the nonanemic group had such an elevation.

DISCUSSION

Anemia in chronic lymphatic leukemia, as in other conditions, must be due to decreased erythropoiesis, increased rate of red cell destruction or blood loss, or combinations of these factors. Since blood loss is rare in chronic lymphatic leukemia, a decrease in the rate of red cell production and/or increase in the rate of red cell destruction are the common causes of anemia in this disease.

1. Analysis of Methods for Measuring the Rates of Red Cell Production and Destruction

A. Rate of red cell production: A semiquantitative estimation of the rate of red cell formation may be made from the number of erythroblasts seen in sections of marrow, provided that there is no maturation arrest or abnormal amount of karyorrhexis and provided that such sections are obtained from an adequate sample of an active area such as the sternal marrow.11 The number of erythroblasts in an adequate sample of an active area of marrow may be used as a rough index of the total amount of erythroblastic tissue.12-16

Since the introduction of radioisotopes, attempts have been made to measure the rate of red cell production by the rate of their incorporation into the red cell hemoglobin.4,17,19
Fig. 6.—Relationship of lymphocyte count in peripheral blood and concentration of lymphatic tissue in the marrow.

Estimation of the rate of red cell formation from the kinetics of tracer Fe$^{59}$ is unreliable because the tracer follows more than one metabolic pathway into several independent pools of iron each of which probably has its own specific rate of entrance and release of iron.$^{20-24}$

There is thus no direct method for accurately measuring the rate of red cell formation. However, in a steady state the rate of red cell production equals the
Fig. 7.—The relationship between concentration of myeloid tissue and lymphatic tissue. The normal range of concentration of myeloid tissue is defined by the shaded area.

Fig. 8.—The relationship between hemoglobin level and concentration of myeloid tissue. The shaded area represents the normal range of myeloid tissue.

rate of red cell destruction. Therefore, the most reliable method now available for estimating the rate of red cell production is determination of red cell volume (or in suitable cases hemoglobin, hematocrit or red cell count) and red cell survival in a patient in a steady state.
B. Rate of red cell destruction: The rate of red cell destruction may be reliably measured only by performance of a red cell survival test.

2. Etiologic Factors in the Development of Anemia

Prior to the availability of radioisotope technics it was generally accepted that replacement of erythroblasts by leukemic cells was the mechanism by which anemia developed. However, since the bone marrow was rarely biopsied, this opinion was based almost entirely on the histopathology of the marrow at autopsy; patients early in the natural history of their disease were not studied. In the present study all the patients (except Case 9 who subsequently became anemic) with a normal hemoglobin level had a normal amount of erythroblastic tissue. The anemic patients had a decreased amount of erythroblastic tissue and the degree of anemia was roughly correlated with the decrease in erythroblastic tissue.

The red cell survival was normal in each patient in both Groups I and II except Case 20, the most anemic patient with a hemoglobin of 9.3 Gm. per cent. This high incidence of normal red cell survival is in apparent disagreement with prior reports upon the red cell survival in chronic lymphatic leukemia. The discrepancy is explained by the following: (1) Most of our patients were studied early in the natural history of their disease. Freymann and Wetherley-Mein also noted that patients with disease of a similar degree of severity had a normal red cell survival. (2) None of our patients had been treated. Treatment with irradiation and mustards has been followed by a decreased red cell survival in chronic lymphatic leukemia. Rosenthal et al. observed autoimmune hemolytic anemia in occasional patients with chronic lymphatic leukemia following treatment with TEM and splenic irradiation. One patient reported by Weinstein developed severe hemolysis detected two months after treatment with P32. Freymann observed a rapid fall in hemoglobin in some cases after the use of Chlorambucil and TEM. Waggener reported that the red cell survival of the patients receiving roentgen therapy was slightly shorter than cases receiving no treatment. It is of interest to note that in one series 25 per cent of the patients treated with P32 or total body spray x-ray developed a hemolytic anemia. A reduction in red cell life has been demonstrated in normal rabbits exposed to irradiation.

Analysis of our data based upon histopathologic observations and upon hemoglobin and red cell survival indicates that the rate of red cell formation is normal in nonanemic patients and decreased in patients who are anemic. A significantly decreased red cell survival was found only in the patient (Case 20) with the most replacement of myeloid by lymphatic tissue. This suggests that an increased rate of red cell destruction occurs only in patients with massive replacement of myeloid by lymphatic tissue and aggravates the anemia which has previously developed because of a decreased rate of red cell production. The rare patient with anemia due to an autoimmune hemolytic mechanism is an exception.

3. Relationship between Anemia and Natural History of Untreated Chronic Lymphatic Leukemia

The data evaluated indicates a correlation between the development of
anemia and anatomic changes in the bone marrow. This data by itself does not demonstrate any relationship between the mechanisms cited and the duration and progression of the disease in an individual patient.

We are now able to recognize a gradual series of stages in the natural history of untreated chronic lymphatic leukemia provided the patient does not die of other causes.²¹,3⁶ The disease is first present in an asymptomatic form without any or at most insignificant hepatomegaly, splenomegaly, lymphadenopathy or anemia. There is only a slight relative and absolute lymphocytosis. The marrow is normal except for a few islands of lymphatic tissue, hardly more than found in normal elderly individuals.²⁵,³⁶ Over the course of years there is gradual enlargement of liver, spleen and nodes accompanied by a lymphocytosis and progressive increase in the amount of lymphatic tissue in the marrow. At this stage there is usually no anemia (excluding patients with a complicating auto-immune hemolytic disease). Finally, anemia develops when there is massive replacement of the myeloid tissue by lymphatic tissue, almost always coinciding with a marked rise in the number of lymphocytes in the peripheral blood. The major differences between patients are the rate of progression of the disease and the stage in which they are first seen. In any case the degree of enlargement of the spleen, liver and lymph nodes, and especially the lymphocyte count, degree of anemia and amount of lymphatic and myeloid tissue in the marrow are a measure of the progression and duration of the disease in a specific patient.

On the basis of the findings in this paper and of what is known of the natural history of patients with untreated chronic lymphatic leukemia, it would appear that there are several stages in the development of anemia in chronic lymphatic leukemia. At first there is no anemia because there is a normal amount of erythroblastic tissue capable of producing red cells at a normal rate and because the rate of red cell destruction is also normal. In the intermediate stage the bone marrow is more extensively infiltrated with lymphatic tissue, the amount of erythroblastic tissue is less than normal and the patients are mildly anemic due to a decreased rate of red cell formation; the rate of red cell destruction is still normal. In the most advanced stage the fat and erythroblastic tissue is almost completely replaced by lymphatic tissue and the decreased rate of red cell formation may be aggravated by an increased rate of red cell destruction. This sequence of events in the development of anemia, decreased rate of red cell formation preceding the increased rate of red cell destruction, is in agreement with the observations of Wetherly-Mein²⁷ and Freymann.²³³

**Summary**

The following conclusions can be made concerning the cause of anemia in patients with untreated chronic lymphatic leukemia excluding the rare case with an autoimmune hemolytic anemia:

1. Early in the natural history of the disease there is a normal amount of erythroblastic tissue which produces red cells at a normal rate. The rate of red cell destruction is also normal.

2. With progression of the disease the erythroblastic tissue is gradually replaced by lymphatic tissue, leading to a decrease in red cell production. The rate of red cell destruction is still normal.
3. Only late in the disease may decreased rate of red cell production be aggravated by an increased rate of red cell destruction.

**SUMMARIO IN INTERLINGUA**

Le sequente conclusiones es permitite con respecto al causa de anemia in patientes con chronic leucemia lymphatic non-tractate, (non inclusive le rar caso de anemia hemolytic autoimmun):

1. A un tempore precoce in le historia natural del morbo il ha un quantitate normal de tissu erythroblastic que produce erythrocytos a un rhythm mo normal. Le rapidate de destruction del erythrocytos es etiam normal.

2. Con le progression del morbo le tissu erythroblastic es reimpbaciate gradualmente per tissu lymphatic, resultante in un decrescimento in le production de erythrocytos. Le rapiditate del destruction del erythrocytos remane normal.

3. Solmente tarde in le historia del morbo pote le decrescite rapiditate del production de erythrocytos esser exacerbate per un accrescite rapidate del destruction de erythrocytos.

**REFERENCES**


17. Wasserman, L. R., Rashkoff, I. A.,
ANEMIA IN CHRONIC LYMPHATIC LEUKEMIA


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