HYPOTHESIS

The Leukemias—Proliferative or Accumulative?

By Howard R. Bierman

The traditional view that leukemia is due primarily to a rapid rate of leukocyte division has directed antileukemic therapy for the past 20 years at destroying or interfering with the alleged rapid DNA and RNA synthesis and abundant mitoses. Without detracting from the noteworthy advances made in conventional chemotherapy, less than 1 per cent of cases of acute leukemia will survive 5 years. Some patients who experience the longest survival receive the least treatment. Studies in the past 20 years seriously question the classical concept of the leukemias, and cancer in general, as diseases primarily of rapid and excessive proliferation. This review supports our original contention that granulocytic leukemia, in particular, and probably other leukemias are disorders of the normal equilibrium between leukocyte production and destruction. Specifically, the rate of individual PMN generation in granulocytic leukemia is similar to, or slower than that in the normal subject; the orderly maturation process of immature granulocytes is defective; and delivery from the tissues to the blood and removal therefrom are impaired. These abnormalities result in an increasing accumulation of a divisible granulocyte pool. The progressive increase in ratio of precursor immature cells to mature leukocytes points to predominantly accumulative rather than proliferative process. The therapeutic attack upon these leukemias therefore need not be aimed at destruction of the alleged rapid proliferative target. Rather a more physiologic approach to reestablish the normal maturation peak and regulatory control of leukopoiesis appears more reasonable (Fig. 1).

REVIEW OF PERTINENT LITERATURE IN THE HUMAN LEUKEMIAS

Normal or Slow Rate of Leukocyte Production

The data indicate that in granulocytic leukemias the rate of production of PMN’s is usually equal to, or slower than, that in the normal subject. The production of PMN’s in normal man varies from 0.71 to 23.70 x 10⁷ PMN’s/Kg./day (Table 1). In chronic granulocytic leukemia, the PMN production rate varies from 0.19 to 7.48 x 10⁷/Kg. body weight/day. Radioisotope data confirm slow leukocyte generation time in acute leukemias with less prolongation in the chronic leukemias. Radioactive labeling of leukocytes does not accurately reflect rates of DNA synthesis of leukocytes.
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Table 1.—Rate of PMN Production X 10⁹/Kg. Body Weight/24 Hours

<table>
<thead>
<tr>
<th>Normal</th>
<th>CGL</th>
<th>CGL &gt; AGL</th>
<th>AGL</th>
<th>Method and Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.86-5.54</td>
<td>0.19-0.97</td>
<td>0.83-7.02</td>
<td>8.03-11.10</td>
<td>Repopulation (1959)</td>
</tr>
<tr>
<td>1.08-12.20</td>
<td>1.00-7.48</td>
<td>0.04-10.40</td>
<td>3.15-4.14</td>
<td>Lifespan (1958, 1960)</td>
</tr>
<tr>
<td>0.71-23.70</td>
<td>0.32</td>
<td>0.01-7.05</td>
<td>—</td>
<td>Leukapheresis (1959, 1960, 1961)</td>
</tr>
<tr>
<td>3.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>RBC Method (1954)</td>
</tr>
<tr>
<td>2.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>Daily Requirement (1962)</td>
</tr>
</tbody>
</table>

synthesis or cell generation time unless all significant portions of the cell populations are uniformly labeled. Less than 30 per cent of the leukocytes actively incorporate tritiated thymidine in patients with acute granulocytic or lymphocytic leukemia. The majority of leukocytes in acute leukemia lie dormant (G₀ pool) without undergoing cell division for days or longer.

Assuming that one can compare the normal and leukemic cell populations, there is a lower labeling of the total leukemic population than in the normal cell population. The earlier cells in the leukemic populations may have lost their ability to divide and thus enter the G₀ pool.

DNA synthesis times for the normal human myelocyte, myeloblast and erythroid precursor in vivo and in vitro are not appreciably different from their leukemic counterparts. The slow mean rate of radioactive labeling and absolute radioactivity of leukemia cells is probably due to an arrest in the S or G₁ periods and their ratio to the G₀ pool.

Prolonged Circulating Lifespan

The circulating lifespan of leukocytes is longer in the leukemic state than in the hematologically normal individual (Table 2). In chronic granulocytic leukemia, a 23 to 26 day PMN population exists which has not been found in the normal subject. As the disease becomes progressively more fulminant, the decreasing PMN populations become shorter-lived (Table 3). Labeled myeloblasts in granulocytic leukemia persist in the circulation much longer than normal granulocytes. In chronic lymphocytic leukemia, Hamilton found lymphocyte lifespans of 85 and 300 days.

Increase of Leukocyte Resistance

The PMN or lymphocyte in their respective leukemias exhibit exceptional resistance to destruction which may contribute to their prolonged lifespans.

Leukodynamics

A delicate balance is normally maintained between the peripheral blood
Table 2.—Postmitotic Lifespan of Leukocytes in Normal Man

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Average Survival Time</th>
<th>Investigator</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulocyte</td>
<td>4 days</td>
<td>Roberts &amp; Kracke (1930)</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>9 days</td>
<td>Ottesen (1954)</td>
</tr>
<tr>
<td>Granulocyte</td>
<td>9 days</td>
<td>Hamilton (1957)</td>
</tr>
<tr>
<td>PMN</td>
<td>8.6 ± 1.7 &amp; 13.6 ± 2.0 days</td>
<td>Bierman (1959)</td>
</tr>
<tr>
<td>WBC</td>
<td>16 days, 50%</td>
<td>Weisberger &amp; Levine (1954)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Weisberger &amp; Sunland (1955)</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>3-4 and 100-200 days</td>
<td>Ottesen (1954)</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>3-4 and 100-200 days</td>
<td>Hamilton (1957)</td>
</tr>
</tbody>
</table>

Table 3.—Comparison of I.R.T. Values—PMN (Bierman et al.9)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. of Studies</th>
<th>IRT</th>
<th>IRT₂</th>
<th>IRT₃</th>
<th>IRT₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>–</td>
<td>8.6</td>
<td>13.6</td>
<td>–</td>
</tr>
<tr>
<td>CGL</td>
<td>7</td>
<td>–</td>
<td>8.4</td>
<td>14.1</td>
<td>23.7</td>
</tr>
<tr>
<td>CGL &gt; AGL</td>
<td>9</td>
<td>4.9</td>
<td>8.9</td>
<td>15.2</td>
<td>18.6</td>
</tr>
<tr>
<td>AGL</td>
<td>3</td>
<td>3.7</td>
<td>7.8</td>
<td>13.6</td>
<td>–</td>
</tr>
</tbody>
</table>

leukocytes and the tissue reservoirs. The marrow compartment contains a higher proportion of immature granulocytes than in the circulation. For every 1000 PMN’s found circulating in the peripheral blood, there are 10 metamyelocytes and one myelocyte (Fig. 2). The marrow differential reveals 1100 metamyelocytes and 950 myelocytes for each 1000 PMN’s (Fig. 2) Table 4).

In acute or chronic granulocytic leukemia, the ratio of PMN’s to metamyelocytes and myelocytes in the peripheral blood is surprisingly similar to that in the marrow (Fig. 2). Removal of leukocytes from the peripheral blood by
Table 4.—Relationship of Granulocytes in Marrow and Peripheral Blood (per cu. mm.)
(Derived from 50 patients with AGL and 50 patients with CGL)

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>CGL</th>
<th>AGL</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Marrow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1100</td>
<td>222</td>
<td>562</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>158</td>
<td>200</td>
</tr>
<tr>
<td>Metamyelocyte</td>
<td>950</td>
<td>644</td>
<td>12,727</td>
</tr>
<tr>
<td>Marrow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1</td>
<td>456</td>
<td>18,600</td>
</tr>
</tbody>
</table>

leukopheresis in the hematologically normal subject is simultaneously accompanied by a prompt release of PMN’s from the reservoirs into the peripheral blood, which generally exceeds that being removed. The peripheral circulating leukocyte concentration remains unchanged or may actually increase. In remarkable contrast, leukopheresis in patients with granulocytic leukemia is accompanied by a prompt decrease in the peripheral leukocyte concentration. The peripheral blood in granulocytic leukemia is apparently divorced from significant replenishment by tissue reservoirs. Apparently the mechanism for discharge of leukocytes from the tissue reservoirs is impaired in granulocytic leukemia. The removal of a large spleen in granulocytic leukemia provokes myeloblastic crises and suggests that splenic function and its products might be involved in leukopoietic control and maturation.

Products of Leukocyte Destruction

Uric Acid. The uric acid levels in serum and urine are elevated in many, but not all, the leukemias. Uricosuria and uricosuria are often employed as evidence of excessive leukocyte destruction. Profound discrepancies, however, are common (Table 5). For example, the 24-hour urinary excretion of uric acid does not increase materially in acute or chronic lymphocytic leukemia,
with a rising count to 141,000, suggesting a decrease in uric acid formation for each leukocyte in these leukemias. The findings in granulocytic leukemia are similar.

**Phosphorus.** Henderson in 1903 demonstrated that phosphorus was commonly retained in the leukemias with or without a negative nitrogen balance. The retention was more extreme as the patient failed, again suggesting an impaired leukocyte destruction and prolonged lifespan of leukocytes in relation to the enlarging leukocyte mass.

**Thoracic Duct Lymph**

Virchow, who originated the proliferative concept of leukemia, claimed that the excessive leukocytes in leukemia made their way via the thoracic duct into the circulation. The leukocyte count of lymph obtained from the thoracic duct in nonleukemic individuals varies from 3000 to 20,000/cu. mm. In lymphocytic leukemia the counts ranged from 2800 to 50,000/cu. mm.
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Table 5.—Uric Acid Production in Leukemia

<table>
<thead>
<tr>
<th>Type</th>
<th>Av. WBC ( \times 10^3 )</th>
<th>Serum U.A. Mg. %</th>
<th>Urine U.A. Mg./Day</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonleukemic</td>
<td>7</td>
<td>4.8</td>
<td>452</td>
<td>Krakoff &amp; Balis (1964)</td>
</tr>
<tr>
<td>CGL (Controlled)</td>
<td>16</td>
<td>5.6</td>
<td>507</td>
<td>Sandberg, et al. (1956)</td>
</tr>
<tr>
<td>ACL</td>
<td>44</td>
<td>3.17</td>
<td>753</td>
<td>Sandberg, et al. (1956)</td>
</tr>
<tr>
<td>ALL</td>
<td>141</td>
<td>11.44</td>
<td>888</td>
<td>Sandberg, et al. (1956)</td>
</tr>
<tr>
<td>CLL</td>
<td>182</td>
<td>5.5</td>
<td>491</td>
<td>Krakoff &amp; Balis (1964)</td>
</tr>
<tr>
<td>CGL</td>
<td>198</td>
<td>8.6</td>
<td>1071</td>
<td>Krakoff &amp; Balis (1964)</td>
</tr>
</tbody>
</table>

Fig. 3.—Regulatory control of granulopoiesis.

The thoracic duct count in lymphocytic leukemia appears to be unrelated to the number of leukocytes circulating in the peripheral blood, and indeed implies recirculation of lymphocytes and not rapid production as subsequently confirmed by Gowans.43

Leukopoietic Stimulants

Leukopoietin G is a water-soluble, nonpyrogenic, small-molecular, non-protein substance which stimulates polymorphonuclear leukocytosis and granulopoiesis in the Wistar rat, CF1 mouse and presumably in man.44-46 Leukopoietin G activity in acute or chronic granulocytic leukemia is similar to that found in hematologically normal subjects44 and is not higher, as would be expected if excessively rapid leukopoiesis were a predominant feature of these leukemias.

Maturation Abnormalities

The limitation of the leukemias usually to a single cell line indicates that the leukemic process acts beyond the progenitor cell at, or later than, the differentiated stem cell level (Fig. 3). Dedifferentiation does not occur. A delicate
homeostatic balance exists in the normal subject, maintaining the steady hematoopoietic state and perpetuating the progenitor and stem cell populations as well. The number of times a stem cell divides to perpetuate the mature progeny is speculative. This homeostatic balance is altered in the leukemias.

In many leukemias the degree of maturation arrest may vary considerably. In the indolent chronic leukemias with a preponderantly mature picture, a significant, although slight, increase in immature leukocytes indicates that the impaired maturation is minimal. The progressive lack of maturation as the leukemia becomes more fulminant or “acute” results in a shift to a more immature cell population. The degree of impairment of maturation becomes both more complete and is expressed at an earlier level—eventuating in a “blastic” hemogram. The lack of maturation without adequate feedback control produces progressively ineffective leukopoiesis (Fig. 3).

The mature PMN apparently exerts some control in acute leukemia. Apparently the feedback mechanism that is involved is related to products within the mature cell which are released in regulated amounts and thereby control the level of leukocyte production and leukocyte level. However, if the mature cell resists breakdown and circulates longer than normal, or is destroyed extravascularly, this feedback control substance therefore fails to reach the receptor site in the marrow in necessary amounts. Consequently, there is an enlargement of the dividing cell population which is independent of the rate of proliferation, resulting in an unrestrained increase in leukocytes.

Eventually, complete impairment of maturation results in no mature cells, and consequently the picture of myeloblastic leukemia without any opportunity for inhibition of leukopoiesis by substances contained in the PMN. Failure to liberate the block in maturation tends further to perpetuate it, which fosters a progressively more immature picture.

A substantial portion of leukocytes remain immature in chronic granulocytic leukemias—in the potentially divisible state. Stem cells, myeloblasts, myelocytes, and metamyelocytes divide more slowly and persist longer than is normal in each stage, further contributing to the abnormally enlarging pool of divisible cells. Some maturation obviously takes place, which accounts for variable numbers of PMN's that are seen with prolonged life-spans in chronic granulocytic leukemia. However, as the disease becomes more fulminant, the proportion of maturation diminishes, and the size of the PMN pool decreases consequent to the proportionate increase in the immature pool.

**Regulatory Control of Leukopoiesis**

Hematodepressive radiation, chemicals, or viruses may damage regulatory components or enzyme-forming mechanisms which control the ratio of stem to mature cell divisions. Regulatory enzymes are generally unstable and can be expected to be bound to an inhibitor-activator for stability. Elucidation of the highly regulated sequence of events from stimulation through each step of the maturation process is consequently difficult.

Stimulators of cell growth are closely related to regulators, probably in the same tissue, perhaps within the same molecular structure. Regulation of each
of the marrow elements must be interrelated in order to maintain the consistent ratio between the erythroid, leukocyte and megakaryocyte populations. This stimulation and inhibition probably operates at both the pluripotential progenitor and unipotential stem cell level, similar to the erythropoietic mechanism.

There is a marked variation in the influence upon cell growth and nucleic acid synthesis by different serums in tissue culture.\textsuperscript{19-51} High molecular fractions (50,000 to 70,000 m.w.) of beef kidney homogenates contain both stimulating and maturation components. Molecular fractions (20,000 m.w. or lower) exhibit primarily the maturation factor. Similar findings occur with molecular fractions of chick embryo extract.\textsuperscript{52}

Leukopoiesis may be regulated by one or many enzymes independently, or more likely in coordinated combinations (concerted inhibition) which control the production of the final mature functioning cell. The more complex the organism or system, the more multiple controls are needed. Full control of leukopoiesis apparently requires multiple substances in critical concentration to influence one or more enzyme steps. The same enzyme may perform different functions at different cell levels, thus emphasizing the delicate control that may be established.

Mature leukocytes in all probability contain multiple inhibitors of leukopoiesis.\textsuperscript{9,32,37,48-53,54} Mature granulocytes from a normal individual or from a patient with chronic granulocytic leukemia will inhibit DNA synthesis by lymphocytes. These inhibitory effects have been shown with intact cells and saline extracts. Perry has reported that thymidine phosphorylase derived from mature granulocytes will inhibit DNA synthesis.\textsuperscript{55} The rate-limiting phosphorylation of thymidine does not as yet have a clear role, but probably influences the large variation in grain counting and chromosome labeling.\textsuperscript{21-47}

**THERAPEUTIC FUTURE OF THE LEUKEMIAS**

*The Prediagnostic Period*

The slow rate of leukocyte accumulation indicates that the leukemic process has been initiated much earlier than previously anticipated.\textsuperscript{66} Consequently, this long preclinical period of years in some instances offers an excellent opportunity to correct the leukemic state before irreparable changes have occurred.

*Influence of Blood and Components*

Spontaneous remissions in acute leukemia have been related to blood transfusions.\textsuperscript{61-63} Fresh blood appears more satisfactory than stored bank blood, perhaps reflecting the instability of this substance.

Fresh whole blood and its fractions in large amounts may produce short remissions\textsuperscript{63,64} (Table 6). The infusion of erythrocytes, plasma and platelets will replace their respective depleted reservoirs, but the infusion of PMN's has caused remissions in acute lymphocytic leukemia—more rapid and dramatic than seen with antimetabolites or alkylating agents. PMN's derived from normal subjects or from patients with chronic granulocytic leukemia are apparently equally effective.\textsuperscript{77} Again, however, these remissions are usually short,
suggesting consumption of the corrective ingredients within 1 to 12 weeks. These observations suggest a possible method of treatment other than interfering with proliferation—that of possibly correcting the basic cellular defect of maturation.

The chronologic sequence of events in such induced remissions in acute lymphocytic leukemia reveal almost simultaneous reappearance of normal granulocytopoiesis in the marrow and blood. The exact quantitative and kinetic significance of these changes remains to be elucidated. These remissions encouragingly suggest that the pathologic accumulation of cells in many patients with leukemia is correctable without cell destructive agents or devices.

**Conclusions**

Contrary to the traditional concept, in many instances the leukemias are characterized by an essentially normal or slower individual leukocyte generation time, impaired maturation, altered cell release into the circulation, and prolonged survival of the “leukemic” cell, resulting in a progressive accretion of the potentially divisible cell population arrested in intermediary phases of development.

We have come to a critical reorientation in our thinking of the leukemias and neoplasia in general. Continued expansion along conventional lines might
be profitably complemented by another program aimed at correction of the leukemic process by encouraging maturation without cellular destruction.

The significance of this moment is the inevitable realization that the dream of a cell specific "magic bullet" which would jam the metabolic machinery of the "leukemic cell" may instead correct that defective mechanism by aiding the leukocyte to mature properly. Efforts to accelerate or correct the altered maturation may offer additional approaches to the treatment of the leukemias with or without conventional chemotherapy. A reevaluation of the leukemias and possibly other neoplasms as disorders primarily of accumulation rather than proliferation may be a useful working hypothesis to encourage newer therapeutic approaches.

SUMMARIO IN INTERLINGUA

In deviacion ab le conception traditional, le leucemias—in multe casos—es characterisate per essentimente normal tempores individual de generation leucocytic, defectos de maturation, alteration del liberation de cellulas ad in le circulation, e un prolongate longevitate del cellulas "leucemic," con le resultato de un accretion progressive del potentialmente divisibile population cellular que ha essite arrestate in phases intermediari de su disveloppamento.

Nos ha arrivate a un puncto de reorientation critic in nostre ideas relative al leucemias e a neoplasia in general. Il pare ben possibile che il va esser profitabile supplementar le continue expansion del methodos conventional per un altere programa visante al correction del processo leucemic per stimulus le maturation del cellulas sin destruer los.

Le signification historic de iste momento es le inevitabile recognition que le ideal de un "shrapnel magic" a specificitate cellular que disruperea le mechanismo metabolic del "cellula leucemic" debe esser reimplaciate o supplementate per le ideal de un agente a specificitate cellular que corrigerea le defective mechanismo metabolic del "cellula leucemic" per asister le leucocyt in normalisar su maturation. Le effortio de accelerar o de corriger le imperfecte maturation del leucocytos va forsan resultar in le disveloppamento de nove methodologias pro le tractamento del leucemias, con o sin le uso de modalitates de chimotherapia conventional. Un re-evalutation del leucemias e possibilemente de altere disordines neoplastic como disordines de accumulation plus tosto que de proliferation duce possibilemente a un utile hypothese de travalo, con le resultato possibile del disveloppamento de nove methodos therapeutic.

REFERENCES


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Hypothesis: The Leukemias—Proliferative or Accumulative?

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