Intravascular Granulocyte Kinetics in Acute Leukemia

By JERRY L. SPIVAK, LEONARD H. BRUBAKER AND SEYMOUR PERRY

Infection is a major cause of death in acute leukemia.1 Although the phagocytic activity of the mature granulocyte in the leukemic patient appears to be unimpaired,2,3 numerous studies have demonstrated a defect in granulocyte mobilization to an inflammatory site.4-6 The response is both delayed and diminished. While granulocytopenia, either secondary to marrow replacement or to chemotherapy, contributes to this defect, the possibility of a kinetic abnormality has not been investigated. Accordingly, the present study was undertaken to examine granulocyte kinetics in acute leukemia using diisopropylfluorophosphate (DF32P) as the granulocyte label.7

Methods

Eleven patients with acute leukemia were studied (Table 1). Disease status was evaluated by the criteria of Acute Leukemia Cooperative Group B.8 Three normal volunteers and five patients with Hodgkin’s disease served as controls (Table 2).9 At the time of study all were hematologically normal as determined by blood and bone marrow examination, and none had organomegaly, fever, pruritis, or was receiving therapy.

Autologous granulocytes were labeled in vitro by withdrawing 500 cc. of whole blood into a sterile, pyrogen-free plastic bag* containing 75 cc. of ACD and adding 16–50 μc. of DF32P (285 μc. per mg. in propylene glycol). The labeled blood was incubated for 45–50 minutes at room temperature with gentle agitation. A 20 cc. aliquot was then removed for cell count, differential count and specific activity determination and the remainder reinfused over a 10–15 minute period.

Duplicate 10 cc. aliquots of peripheral blood were drawn for the determination of leukocyte specific activity at 15 minutes, 1 hour, 3 hours, 6 hours, 10 hours, and 24 hours, after reinfusion of the labeled cells. Leukocytes were isolated by a modification of the dextran sedimentation and hypotonic lysis technic as previously described.10 Leukocyte clumping did not occur and cell counts were done using an electronic cell counter.* The cells were then centrifuged to obtain compact cell buttons. These were dissolved in 0.5–1.0 cc. of NCS† reagent and after the addition of 15 cc. of toluene phosphor scintillation fluid, radioactivity was determined in a liquid scintillation spectrometer.† Quenching was monitored by the external standard method. Leukocyte specific activity was expressed as counts per minute per 107 cells.

The expected leukocyte specific activity in the blood at the completion of the infusion of labeled cells was calculated by the method of Mauer et al.11 Blood volume was assumed to be 2.68 L per m2 of body surface area12 which was estimated by the method of Sendroy and Cecchini.13 The experimentally determined leukocyte specific activity at each time interval expressed as a per cent of the expected leukocyte specific activity was plotted against...
time on semilogarithmic paper. The half time for disappearance of the labeled granulocytes was determined from the slope of a line fit to the semilogarithmic points by the least squares method. Except in the three granulocytopenic patients (L.C., C.G., M.C.), who had a low proportion of mature granulocytes, the separation procedure resulted in an increase in granulocyte concentration as compared to that in the blood, so that the final suspension contained 80–90 per cent granulocytes. This differential count remained relatively constant for the

### Table 1.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>WBC/mm³</th>
<th>Differential+</th>
<th>T½ (hours)</th>
<th>Disease Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>W.J.</td>
<td>♂</td>
<td>26</td>
<td>AML*</td>
<td>3,900</td>
<td>46% P</td>
<td>8.5</td>
<td>5.1 Remission; on no therapy</td>
</tr>
<tr>
<td>R.A.</td>
<td>♂</td>
<td>41</td>
<td>AML</td>
<td>5,900</td>
<td>70% P</td>
<td>4.7</td>
<td>10.2 Remission; on daily cytosine arabinoside</td>
</tr>
<tr>
<td>C.H.</td>
<td>♂</td>
<td>49</td>
<td>AML</td>
<td>5,300</td>
<td>50% P</td>
<td>14.0</td>
<td>9.6 Remission; on no therapy</td>
</tr>
<tr>
<td>C.S.</td>
<td>♂</td>
<td>20</td>
<td>ALL †</td>
<td>10,900</td>
<td>86% P</td>
<td>15.4</td>
<td>10.5 Relapse; on no therapy</td>
</tr>
<tr>
<td>W.T.</td>
<td>♂</td>
<td>28</td>
<td>AML</td>
<td>10,900</td>
<td>80% P</td>
<td>7.1</td>
<td>13.7 Remission; on vincristine, prednisone, 6-MP methotrexate</td>
</tr>
<tr>
<td>L.C.</td>
<td>♂</td>
<td>50</td>
<td>AML</td>
<td>2,300</td>
<td>13% P</td>
<td>7.8</td>
<td>9.7 Untreated</td>
</tr>
<tr>
<td>C.G.</td>
<td>♂</td>
<td>46</td>
<td>AML</td>
<td>5,000</td>
<td>25% P</td>
<td>20.5</td>
<td>13.9 Untreated</td>
</tr>
<tr>
<td>W.C.</td>
<td>♂</td>
<td>24</td>
<td>AML</td>
<td>11,600</td>
<td>90% P</td>
<td>25.4</td>
<td>15.5 Remission; on vincristine, prednisone, 6-MP, methotrexate</td>
</tr>
<tr>
<td>C.B.</td>
<td>♂</td>
<td>20</td>
<td>ALL</td>
<td>4,500</td>
<td>85% P</td>
<td>9.2</td>
<td>8.4 Remission; on daily methotrexate</td>
</tr>
<tr>
<td>D.P.</td>
<td>♂</td>
<td>35</td>
<td>ALL</td>
<td>5,100</td>
<td>72% P</td>
<td>11.0</td>
<td>15.7 Remission induction, vincristine, prednisone, splenectomy</td>
</tr>
<tr>
<td>M.C.</td>
<td>♂</td>
<td>16</td>
<td>AML</td>
<td>219,000</td>
<td>13% P</td>
<td>20.8</td>
<td>— Hydroxyurea</td>
</tr>
</tbody>
</table>

*AML—Acute Myelocytic Leukemia.
†ALL—Acute Lymphocytic Leukemia.
P—Polymorphonuclear leukocyte
L—Lymphocyte
E—Eosinophil
Bl—Blast
Prom—Promyelocyte
My—Myelocyte
Meta—Metamyelocyte
samples of individual patients. Consequently, leukocyte specific activity was not corrected for the absolute granulocyte count.

Results

Control Subjects:
Evaluation of the method used for determination leukocyte specific activity revealed that it was quite reproducible. The coefficient of variation of duplicate

Fig. 1.—Disappearance curves of autologous granulocytes in two normal subjects and two patients with Hodgkin's disease.
aliquots processed separately varied between 3 and 7 per cent except for the subject P.H. in whom it was 13 per cent. The leukocyte disappearance curves of the normal volunteers and the patients with Hodgkin's disease were identical (Fig. 1). The half disappearance times in these subjects had a mean of 7.2 hours (Table 2) which is similar to the value of 6.7 hours reported by Cartwright et al.\textsuperscript{14} and 6.4 hours reported by Galbraith et al.\textsuperscript{15} but longer than the 3.8 hour half life of Alexanian et al.\textsuperscript{16}

**Leukemic Subjects**

In this group, the coefficient of variation of duplicate aliquots ranged from 3 to 12 per cent except for patients R.A. and C.H. who had values of 14 and 19 per cent respectively. Thus the experimental method was comparable in both controls and leukemic patients.

The leukemic group was composed of 8 patients with AML and 3 with ALL. None of the patients was febrile at the time of study but 6 patients were receiving treatment (Table 1). In the majority of patients, the granulocyte disappearance curves were complex. Examples are shown in Figure 2. These curves were characterized by an initial decline over the first 3 hours, followed by a shallow plateau during the next 6 hours with a subsequent decline of specific radioactivity by 24 hours after reinfusion of the labeled cells. The disappearance patterns were similar in AML and ALL, untreated (patients L.C.

### Table 2.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>WBC/mm(^3)</th>
<th>Differential *</th>
<th>(T_{1/2}) (hours)</th>
<th>Disease Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.L.</td>
<td>♀</td>
<td>22</td>
<td>Hodgkin’s Disease</td>
<td>6,500</td>
<td>76 P 22 L</td>
<td>6.6 7.0</td>
<td>II B</td>
</tr>
<tr>
<td>R.B.</td>
<td>♂</td>
<td>27</td>
<td>Hodgkin’s Disease</td>
<td>5,000</td>
<td>76 P 17 L 2 Mo</td>
<td>6.5 6.0</td>
<td>II B</td>
</tr>
<tr>
<td>L.H.</td>
<td>♂</td>
<td>46</td>
<td>Hodgkin’s Disease</td>
<td>8,900</td>
<td>72 P 20 L 5 Mo</td>
<td>4.0 6.1</td>
<td>III B</td>
</tr>
<tr>
<td>L.B.</td>
<td>♂</td>
<td>26</td>
<td>Hodgkin’s Disease</td>
<td>5,500</td>
<td>86 P 12 L 2 E</td>
<td>7.6 6.6</td>
<td>I A</td>
</tr>
<tr>
<td>F.C.</td>
<td>♂</td>
<td>63</td>
<td>Hodgkin’s Disease</td>
<td>7,900</td>
<td>80 P 13 L 7 E</td>
<td>8.7 8.9</td>
<td>I A</td>
</tr>
<tr>
<td>R.P.</td>
<td>♂</td>
<td>25</td>
<td>Normal</td>
<td>5,300</td>
<td>54 P 43 L 3 Mo</td>
<td>6.5 8.1</td>
<td></td>
</tr>
<tr>
<td>P.H.</td>
<td>♂</td>
<td>28</td>
<td>Normal</td>
<td>7,600</td>
<td>68 P 30 L 2 E</td>
<td>6.0 7.5</td>
<td></td>
</tr>
<tr>
<td>F.B.</td>
<td>♂</td>
<td>33</td>
<td>Normal</td>
<td>5,900</td>
<td>63 P 36 L 1 E</td>
<td>6.4 7.7</td>
<td></td>
</tr>
</tbody>
</table>

P—Polymorphonuclear Leukocyte, L—Lymphocyte, E—Eosinophil, Mo—Monocyte.
Fig. 2.—Abnormal disappearance curves of autologous granulocytes in four subjects with acute leukemia.

and C.G.), in a patient in relapse (C.S.) and in all the patients in remission except for patient W.J. (Fig. 3). In this individual, the curve appeared normal. Treatment did not appear to affect the configuration of the curves except that with corticosteroid therapy, granulocyte specific activity at 24 hours was increased (Fig. 2, patient W.T.).

Because of the complex nature of these curves, estimation of the granulocyte half disappearance time can only be an approximation. However, in all patients except W.J., whose curve was similar in shape to the controls, the granulocyte
disappearance time of labeled granulocytes was similar to the control groups (Table 1).

**Discussion**

The intravascular disappearance curve of DF$^{32}$P labeled granulocytes represents a summation of the influx of unlabeled cells from the bone marrow and the efflux of labeled and unlabeled cells from the intravascular space. This curve is exponential indicating a random loss of labeled cells from the blood. There is evidence that granulocytes, after once having left the vascular tree, are unable to re-enter in any significant numbers.

In the present study, the granulocyte disappearance curves in our control group were similar to those previously reported. In contrast to the control subjects, ten of the 11 patients with acute leukemia had granulocyte disappearance curves which were different from normal. The disappearance patterns of labeled granulocytes in the leukemic patients were complex and the rates of disappearance were prolonged. This was true for patients in remission as well.
as in untreated patients. Concomitant treatment did not change the overall shape of the disappearance curves. Patients with ALL had curves similar to those with AML but the data are inconclusive in this regard since only three patients with ALL were studied.

There are several possible explanations for the prolonged granulocyte disappearance rates found in the leukemic subjects. A decrease in marrow granulocytes production, either due to tumor or to chemotherapy could be responsible. This possibility appears to be a valid consideration for patients in remission as well as those with marrow infiltration since abnormal etiocholanolone stimulation tests have been found in patients in hematologic remission. A second reason for the prolonged survival of labeled granulocytes in the leukemic patients would be the presence of labeled circulating immature cells. Cartwright and Wintrobe have shown a positive correlation between the half disappearance time for granulocytes in chronic myelocytic leukemia (CML) and the presence of such immature cells. Such a possibility is unlikely in the present study since less than 1 per cent of such cells were seen in 200 cell differentials in the patients with AML in remission and such cells would not be expected in patients with ALL.

Recirculation of the labeled cells could also account for the prolonged disappearance rates. In normal individuals, a recycling of labeled cells has not been found. In CML, however, the spleen appears to act as an extravascular pool from which labeled cells can re-enter the blood and this may be true in chronic lymphocytic leukemia as well. Such a phenomenon appears unlikely in the individuals in this study since there was little splenic enlargement even in the untreated patients. Finally, a complex disappearance curve was also present in one patient who had undergone splenectomy.

As mentioned above, there is no evidence for recirculation of labeled cells, or increased numbers of immature circulating cells to account for the deviation from normal. However, it is possible that the complex disappearance patterns reflect an unsteady state. In normal subjects, granulocyte counts may fluctuate widely and the range of values for the total blood granulocyte pool and granulocyte turnover rate is large. It is also well-documented that granulocyte production and release is not orderly in both AML and ALL. Consequently, the abnormal disappearance curves observed in these studies may be due to such factors. However, their presence in patients in remission is not entirely explained on this basis, particularly since a normal pattern was observed in one of the patients in this study who was in remission.

There have been a number of reports of defective granulocyte mobilization to an inflammatory site in acute leukemia. In several of the patients studied there were normal or increased numbers of circulating mature neutrophils, yet the actual quantity of cells mobilized was low. Recently, similar studies performed on leukemic patients in remission have also demonstrated a defect in granulocyte mobilization. While it is possible that the defective mobilization is due to inadequate bone marrow reserves, it may also be due to inability of granulocytes to leave the blood stream. Such an abnormality could produce the disappearance curves seen in this study.

The possibility of defective granulocyte migration as a cause of the kinetic
abnormalities found in this study is not without precedent. Galbraith, using cross transfusion technics in patients with CML, demonstrated that both an intrinsic leukocyte abnormality and environmental factors were responsible for abnormal granulocyte kinetics in these patients. Since similar studies have not been performed in acute leukemia, the role of either intrinsic cell or environmental abnormalities remains speculative. However, better definition of this problem is important in view of the increasing use of isologous granulocyte transfusions in the treatment of infection in acute leukemia.

**SUMMARY**

Intravascular granulocyte kinetics in 11 patients with acute leukemia and 8 hematologically normal individuals were studied following the infusion of autologous leukocytes labeled in vitro with diisopropylfluorophosphate (DF32P). The kinetics of the labeled granulocytes in the control subjects was similar to that reported in previous studies. Ten of eleven leukemic subjects, however, had abnormal granulocyte disappearance curves and prolonged granulocyte disappearance rates. This was true for patients in remission as well as for untreated patients. The abnormal granulocyte kinetic patterns demonstrated here are in keeping with the disorderly and diminished granulocytopoiesis and defective granulocyte mobilization found in acute leukemia.

**SUMMARIO IN INTERLINGUA**

Esseva studiate le cinetica de granulocytos intravascular in 11 patientes con leucemia acute e 8 subjectos hematologicamente normal post le infusion de autologe leucocytos marcate in vitro con diisopropylfluorophosphato (DF32P). Le cinetica del marcate granulocytos in le subjectos de controlo esseva simile a illo reportate in previe studios. Del altere latere, 10 del 11 subjectos leucemic habeva anormal curvas de disparition de granulocytos e relentate ratas de disparition de ille cellulas. Isto valeva tanto pro patientes in remission como etiam pro nontractate patientes. Le anormal configurationes in le cinetica granulocytic hic demonstrate es congrue con le disordinate e diminuite granulocytopoiese e le defective mobilisation de granulocytos trovate in casos de leucemia acute.

**ACKNOWLEDGMENT**

The authors wish to acknowledge the help of Dr. John Cart, Biometry Branch, National Cancer Institute in the analysis of the data and the technical assistance of Miss Ada Brooks. We are also grateful to Dr. Hans Blom, Walter Reed Army Medical Center for permitting us to study several of his patients. The cooperation of Dr. Paul Carbone, Chief, Medicine Branch, National Cancer Institute, and Dr. Edward S. Henderson, Head, Leukemia Service, Medicine Branch, National Cancer Institute, and Dr. Arthur Serpick, Baltimore Cancer Research Center, is also acknowledged with appreciation.

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