An Unusual Pattern of Neutrophil Kinetics in Sickle Cell Anemia

By Dane R. Boggs, Fred Hyde, and Carl Srodes

Blood neutrophil kinetics were studied with DF$^{32}$P in patients with sickle cell anemia. Neutrophil concentration in venous blood samples and the size of the circulating granulocyte pool (CGP) were high normal or abnormally high in all patients. The total blood granulocyte pool (TBGP) was not increased to the same degree as the CGP so that in part, the neutrophilia associated with sickle cell anemia represents a shift from marginated to circulating blood compartments. The half disappearance time ($T_{1/2}$) of blood neutrophils tended to be short so that with an elevated or high normal TBGP the absolute granulocyte turnover rate (GTR) tended to be increased. The fractional GTR was regularly increased. In most types of neutrophilia the $T_{1/2}$ tends to be increased rather than shortened and the fractional GTR may be normal, decreased, or increased. The increased fractional GTR observed in sickle cell anemia suggests that the absolute increase in neutrophil loss from the blood is not merely a function of increased pool size as was suggested previously.

Neutrophilia is a common finding in a variety of acute or chronic hemolytic anemias. Studies of the kinetic mechanism of neutrophilia of various causes indicate three basic mechanisms or combinations thereof by which an increased concentration of neutrophils in venous blood can be induced: (1) demargination of intravascular neutrophils so that the circulating granulocyte pool (CGP) is increased at the expense of the marginal pool (MGP), but without any increase in the total blood granulocyte pool (TBGP), (2) acceleration of the rate at which neutrophils are released from the storage pool of the marrow to the blood, and (3) reduction in the rate at which neutrophils leave the blood to enter tissues and body cavities. We undertook kinetic studies in the severe, chronic hemolytic anemia, sickle cell anemia, in order to delineate the mechanism of neutrophilia which is a common concomitant of the disease. An unusual kinetic pattern was observed which has implications concerning the mechanism controlling blood neutrophil level.

From the Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Submitted June 20, 1972; accepted July 15, 1972.

These studies were supported in part by a research grant (AM 14352) from the National Institute of Arthritic and Metabolic Diseases and by a Clinical Research Unit grant (F.R.-00056) from the National Institutes of Health.

Dane R. Boggs, M.D.: Professor of Medicine; Chief, Division of Hematology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. Fred Hyde, M.D.: Fellow in Hematology, University of Pittsburgh School of Medicine, Pittsburgh, Pa. Carl Srodes, M.D.: Fellow in Hematology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.

Blood, Vol. 41, No. 1 (January), 1973
MATERIALS AND METHODS

The patients were adults with homozygous sickle cell anemia, documented by hemoglobin electrophoresis and by virtually complete sickling of erythrocytes when exposed to metabisulfite in vitro. Informed consent for the studies was obtained and the studies were carried out in the Clinical Research Unit. All patients were in a relatively good state of health and none had suffered from any form of crisis in the month preceding the study. Two had open, but uninfected leg ulcers at the time of study.

Blood neutrophil concentration was determined from an electronic total leukocyte count and a 200-cell differential count. Blood leukocytes were labeled with radioactive diisopropylfluorophosphate (DF32P) by the original technique described5,6 and slightly modified2 by Athens et al. In brief, approximately 500 ml of whole blood is removed, incubated with DF32P, and returned to the donor. From the determined neutrophil concentration and leukocyte specific activity (CPM/mg leukocyte nitrogen) of the infused blood and the first post-infusion sample, the size of the TBGP is calculated by dilution principal. Periodic blood samples are obtained for 24 hr following infusion from which the disappearance curve and T ½ of blood leukocyte specific activity are determined. The absolute granulocyte turnover rate is calculated from the TBGP and the T ½.

RESULTS

Baseline hematologic data and results of nine DF32P kinetic studies in eight patients are shown in Table 1. Blood neutrophil concentration was high normal in three and above the limits for normal subjects studied with DF32P in six. A representative study is illustrated in Fig. 1. In that study the only value outside 95% confidence limits of normal is the elevated circulating granulocyte pool (CGP). The total blood granulocyte pool (TBGP) is high normal and more than 80% of the TBGP is in the CGP so that the marginal pool (MGP) is low normal. The T ½ is slightly faster than the normal mean so that the high normal TBGP and low normal T ½ yield a granulocyte turnover rate

Table 1. Hematologic and DF32P Kinetic Data in Patients With Sickle Cell Anemia

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sex</th>
<th>Age</th>
<th>Hematocrit (%)</th>
<th>Leukocyte concentration (10⁶ cells/mm³)</th>
<th>Neutrophil concentration (10⁶ cells/mm³)</th>
<th>TBGP (10⁶ cells/kg)</th>
<th>CGP (10⁶ cells/kg)</th>
<th>T ½ (hr)</th>
<th>GTR (10⁶ cells/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.M.</td>
<td>F</td>
<td>43</td>
<td>24</td>
<td>9,600</td>
<td>6,000</td>
<td>64</td>
<td>58</td>
<td>5.2</td>
<td>203</td>
</tr>
<tr>
<td>E.M.</td>
<td>F</td>
<td>16</td>
<td>27</td>
<td>11,900</td>
<td>6,400</td>
<td>87</td>
<td>42</td>
<td>4.6</td>
<td>300</td>
</tr>
<tr>
<td>S.M.</td>
<td>F</td>
<td>19</td>
<td>28</td>
<td>15,800</td>
<td>8,800</td>
<td>76</td>
<td>54</td>
<td>4.4</td>
<td>288</td>
</tr>
<tr>
<td>G.W.</td>
<td>M</td>
<td>24</td>
<td>20</td>
<td>10,300</td>
<td>6,300</td>
<td>67</td>
<td>52</td>
<td>3.6</td>
<td>310</td>
</tr>
<tr>
<td>M.B.</td>
<td>F</td>
<td>32</td>
<td>22</td>
<td>13,800</td>
<td>6,900</td>
<td>167</td>
<td>68</td>
<td>7.0</td>
<td>396</td>
</tr>
<tr>
<td>L.H.</td>
<td>F</td>
<td>23</td>
<td>24</td>
<td>17,800</td>
<td>11,200</td>
<td>94</td>
<td>104</td>
<td>5.2</td>
<td>299</td>
</tr>
<tr>
<td>S.F.**</td>
<td>F</td>
<td>40</td>
<td>37</td>
<td>13,000</td>
<td>10,500</td>
<td>84</td>
<td>70</td>
<td>5.5</td>
<td>254</td>
</tr>
<tr>
<td>R.W.(1)</td>
<td>M</td>
<td>23</td>
<td>23</td>
<td>11,800</td>
<td>8,800</td>
<td>117</td>
<td>70</td>
<td>4.2</td>
<td>435</td>
</tr>
<tr>
<td>(2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal values*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63</td>
</tr>
<tr>
<td>95% confidence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>163</td>
</tr>
</tbody>
</table>

*From Ref. 2.
**See text for details of blood transfusions.
Fig. 1. A representative study of neutrophil kinetics in a patient with sickle cell anemia. Value in parentheses are mean normal values.²

(GTR) in the high normal range. This pattern was characteristic for virtually all studies (Table 1) and the relationship of these values to normal is illustrated in Fig. 2.

Note that the only value consistently above normal limits is the CGP (Fig. 2). The TBGP was above the normal mean in 8/9 studies, but outside 95% confidence limits in only one-third. In all but one patient more than half of the blood neutrophils were circulating and in one (L.F.) no marginal pool could be measured. The T½ was virtually normal in one patient, but was below the normal mean in 8/9 studies. The GTR was above the normal mean in all patients.

The degree of anemia and the per cent S hemoglobin present had no apparent relationship to the neutrophil kinetic pattern. Two patients (R.W. and S.F.) were transfused to a normal or near normal hematocrit level as part of a study designed to determine whether correction of anemia is of benefit in the treatment of leg ulcers. In both patients the percent A hemoglobin exceeded 90% after transfusion. The kinetic data in R.W. obtained before and 3 wk after transfusion are virtually identical (Table 1). Similarly, data from S.F., obtained after transfusion, were quite similar to that from other, nontransfused patients.

DISCUSSION

Neutrophilia in sickle cell anemia is, at least in part, due to a shift of neutrophils from the marginal pool (MGP) to the circulating pool (CGP). The degree of neutrophilia suggested by determining neutrophil counts in venous blood samples is somewhat exaggerated as compared to the degree of neutrophilia as judged by the size of the total blood granulocyte pool (TBGP). Half of blood neutrophils are in the CGP in the average normal subject, but the variation for this figure in normals is so great that little can be concluded from study of a single patient.² However, more than half of blood neutrophils were circulating in 8/9 studies in sickle cell anemia which suggests significant deviation from normal in this regard.
Fig. 2. Distribution of values for total blood granulocyte pool (TBGP), circulating granulocyte pool (CGP), half disappearance time of labeled cells from the blood (T½), and granulocyte turnover rate (GTR) in patients with sickle cell anemia as compared to normal subjects.2

Taken as a group the TBGP tended to be increased and the T ½ short so that the granulocyte turnover rate (GTR) also tended to be increased. This indicates that in addition to demargination, increased production and increased utilization of neutrophils is taking place in the patient with sickle cell anemia. The validity of comparing results in these patients to results previously collected in normal subjects can be questioned. Most of the normal volunteers were white and it is possible that there is a different pattern of blood neutrophil kinetics in white as compared to black populations. However, there is a tendency for blood neutrophil levels to be lower in black than in white.7 That observation suggests the differences from normal observed in the present study, particularly with respect to pool size, may be even greater than indicated by the data.

The reason for abnormal neutrophil kinetics is not immediately apparent. Adults with sickle cell anemia usually have undergone an “autosplenectomy.”1 In dogs studied before splenectomy and again some time after splenectomy, there was no significant change in neutrophil kinetics.8 We have studied a few patients splenectomized months or years before for idiopathic thrombocytopenia or autoimmune hemolytic anemia in whom neutrophil kinetics were quite normal. Thus, it seems unlikely that “autosplenectomy” contributes significantly to the abnormality. Anemia with vasodilation and increased blood flow might explain demargination and it can be suggested that increased neutrophil utilization might be part of the intravascular sickling process. However, these explanations are unlikely since kinetic changes in the two patients transfused to normal levels and in whom more than 90% of circulating red cells were normal were similar to the anemic group. Determining whether this pattern is peculiar to sickle cell anemia or is characteristic of the neutrophilia observed in other forms of chronic hemolysis will require further study.

In most other forms of neutrophilia the T ½ is prolonged rather than shortened as in the present study. In neutrophilia due to infection or to a variety of miscellaneous causes the increased GTR is best correlated with the size of the TBGP (Fig. 3).2 This correlation is poor, if present at all in sickle cell anemia (Fig. 3). The difference in the kinetic pattern of the neutrophilia of
Fig. 3. Relationship between the total blood granulocyte pool and granulocyte turnover rate. Data on patients with chronic infection (17 patients) and miscellaneous causes of neutrophilia (idiopathic leukocytosis, seven patients; Hodgkin's disease, seven patients; pulmonary infiltrate of unknown cause, three patients; and one patient with giant follicle lymphosarcoma) have been published previously. The shaded area encompasses all non-sickle patients.

sickle cell anemia and other forms of chronic neutrophilia is further illustrated by the comparisons shown in Fig. 4. In other forms of neutrophilia the fractional blood granulocyte turnover rate (per cent of the TBGP turning over per unit time as opposed to the absolute turnover rate) is variably changed and is about as likely to be decreased as it is to be increased. However, the fractional turnover rate was markedly increased in all studies of sickle cell anemia (Fig. 4). A consistent elevation of the fractional GTR has implications for the manner in which levels of blood neutrophils are regulated.

The previous observations of a correlation of the absolute GTR with the TBGP but without any consistent change in the fractional GTR suggested that increased delivery rate of granulocytes to tissue was accomplished primarily by increasing pool size. In such a system the number of neutrophils passing an exit point which are removed from the blood need not be changed. The present study indicates that in sickle cell anemia the proportion of those passing an exit point which are lost from the blood is increased, as judged by the consistent increase in fractional GTR (Fig. 4).

The feedback loop which controls the rate of release of neutrophils from the bone marrow storage pool could be geared to blood pool size and/or to tissue demand for neutrophils and rate of neutrophil loss to tissue. Control by pool size alone is an untenable hypothesis since such a system could not sustain neutrophilia with an increase in CGP and MGP.

Loss of neutrophils from the blood can be divided into a "passive" normal process and an "active" process superimposed by inflammation or other pathological processes. In normal subjects the passive process could predominate. In this circumstance a neutrophil transversing the capillaries and post-capillary venules of normal tissue, such as the lung, stands a fixed probability, $K_i$, of diapedesis. This diapedesis could be a function of a normal chemotactic demand in that tissue, a property of the neutrophil, or combinations thereof. In this circumstance the total number of neutrophils leaving the blood each day is also a function of the fraction of the TBGP which is in the proper tissue ($K_s$); total neutrophils leaving blood = $K_i \times K_s \times TBGP$. It seems apparent
that a neutrophil must be marginated before it can diapedese; so the MGP/CGP ratio in the area is also important.

Active cell loss occurs when some event, such as infection or inflammation, occurs in tissue, signaling an increased demand for neutrophil diapedesis in that area. Observation of developing inflammation in living tissue such as the rabbit ear chamber indicates increased diapedesis is accompanied by a marked increase in the total number of marginated cells in the area, i.e., both Kt and Ks are increased.

A loop geared solely to cell demand by tissues can control the system but is somewhat tenuous as an explanation for the marked variation in fractional GTR in circumstances where the absolute GTR is increased. Furthermore, it fails to explain observations of increased release of neutrophils from perfused marrow if the perfusate was neutrophil poor as compared to neutrophil rich.

Thus, we would suggest that there may be two classes of feedback loops regulating release of cells from marrow. One is regulated by blood pool size, a passive loop, and the second is geared to demand for neutrophils in an abnormal area such as an inflammation, an active loop. The presumed humoral mediator could be the same for both loops. However, the pool size loop could, in theory, be geared to the number of cells in marrow sinuses rather than being a humoral stimulus.

Fig. 4. Changes in fractional blood granulocyte turnover rate (GTR) in neutrophilia of sickle cell anemia, infection, and miscellaneous conditions (see legend to Fig. 3 for details of patient population). The absolute GTR is plotted against T\(^{1/2}\), both as fractions of mean normal values. If fractional GTR was unchanged, values should fall on the diagonal dotted line, values above that line represent an increase in fractional GTR, and values below a decrease.
In neutrophilia the passive loop would be inactive due to increased pool size, but neutrophilia would be maintained until the reason for activation of the active loop had disappeared. In this circumstance the increase in absolute GTR would be a function of tissue demand and the nature of the change in fractional GTR would reflect the degree of overshoot induced in blood pool size by the initial activation of the active loop. If pool size was expanded beyond the size required to meet the demands of tissue, the fractional GTR would be less than normal. If an overshoot was not induced or was no longer present, an increased fractional GTR would be observed.

REFERENCES
An Unusual Pattern of Neutrophil Kinetics in Sickle Cell Anemia

Dane R. Boggs, Fred Hyde and Carl Srodes