Erythropoietin and the Regulation of Erythropoiesis in DiGuglielmo’s Syndrome

By J. W. ADAMSON AND C. A. FINCH

Observations relating to the regulation of red cell production in four patients with DiGuglielmo’s syndrome and one with malignant lymphoma are presented. Erythropoiesis in these patients was characterized by bizarre forms, marked erythroid hyperplasia and ineffective erythropoiesis. Erythropoietin excretion was elevated, in keeping with the degree of anemia. Hypertransfusion of these patients resulted in a marked decrease in erythropoietin excretion and a reduction in marrow erythroid hyperplasia although morphologic abnormalities of the individual cells in both granulocytic and erythroid cell lines remained. The alteration in marrow function in response to transfusions was reflected by the E:G ratio and in iron kinetic and reticulocyte measurements which demonstrated a decline in both total and effective erythropoiesis. It is concluded that proliferation of the erythroid marrow in these patients is under physiologic regulation and that the defects observed are expressed during cell maturation.

DIGUGLIELMO’S SYNDROME (erythroleukemia, erythremic myelosis) is characterized by a morphologically bizarre marrow, erythroid hyperplasia with ineffective erythropoiesis, unresponsiveness to hematinic agents, variable involvement of myeloid precursors and megakaryocytes, and a tendency to terminate in acute granulocytic leukemia if death is not brought about sooner by infection, complication of transfusions or bleeding. The clinical course is unpredictable. This disorder has been generally classified by workers as part of the myeloproliferative syndrome or as representing a primary malignancy peculiar to the erythroid series. The latter view, originally offered by Copelli and DiGuglielmo, was based primarily on morphologic considerations. Limited insight into the physiology of this disorder has been provided by morphologic means, however, and information is only now becoming available with regard to the control of red cell production in DiGuglielmo’s syndrome. This report details the effects of hypertransfusion studies in four patients with so-called DiGuglielmo’s syndrome, one of whom formed the basis of a previous report, and correlates changes in marrow function with the physiological mechanisms controlling red cell production.
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morphology with erythropoietin excretion and iron kinetics. In addition, similar studies are reported in a patient with well-differentiated lymphosarcoma associated with bizarre and ineffective erythropoiesis.

MATERIALS AND METHODS

The patients studied were admitted to the Clinical Research Center of the University Hospital or to the Medical Service of the King County Hospital, Seattle, Wash. All were anemic and prior to study had required transfusions in order to maintain an adequate hemoglobin. After an initial period in which baseline parameters of marrow function were obtained, the hematocrit of each patient was raised to and maintained at normal levels by exchange transfusions using packed red blood cells. This procedure was well tolerated in all subjects.

Hematologic measurements including hematocrit, reticulocyte count, WBC and platelet count were made using standard techniques. Bone marrow aspirations were performed on admission and at intervals following the transfusion procedures. Marrow smears were stained both by a modified Romanowsky technic and with Prussian blue and estimates of the total marrow cellularity and erythroid: granulocytic (E:G) ratio made from the clot section and stained smears, respectively. At least 1000 intact cells were counted to determine the E:G ratio.

The regulation of erythropoiesis was assessed by a number of parameters. Plasma iron turnover, erythrocyte iron turnover and per cent utilization of radioiron were measured as previously described.9 The patients' sera were labeled with 5–10 µCi of 59Fe citrate and the labeled sera then injected intravenously. The immediate disappearance rate and the amount of radioiron appearing in the red cell mass at 14 days were determined. The initial ferrokinetic studies were performed at least 1 week prior to exchange transfusion to preclude any effect of the administered red cells on the eventual incorporation of the radioiron. Subsequent iron turnover studies were performed 2–7 days after the last transfusion procedure in all of the patients. Frequent reticulocyte counts were obtained before and after phlebotomy. The reticulocytes in 10,000 red cells were enumerated using a Miller ocular9 and the counts obtained were corrected for the hematocrit in order to estimate the index of effective marrow production.8 Urine was collected for erythropoietin determinations for several days both before and following hypertransfusion. The method of concentration of the urine and quantitation of erythropoietin was carried out as previously described.10,11

RESULTS

Initial hematologic values for each patient are shown in Table 1. All were anemic with hematocrits ranging from 21.5 to 32.8 per cent. At the time of admission, bone marrow cellularity was moderately to markedly increased in each subject; differential counts of smears of marrow particles indicated that the hypercellularity was predominately, if not solely, due to increased numbers of erythroid precursors.

The erythroid elements were highly abnormal and showed varying degrees of megaloblastic change. Cells in mitosis and multinucleated cells were common (Fig. 1). Staining of marrow cells with benzidine12 demonstrated the presence of hemoglobin when the identity of giant multinucleated forms was in question. Abnormal sideroblasts, including ringed forms, were seen on bone marrow smears stained for iron in four of the five patients. With the exception of patient L.R., abnormalities were also seen in granulocytic maturation and were characterized by megaloblastic changes and by a shift to the left in the maturation sequence. Auer rods were found in myeloblasts of pa-
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Fig. 1.—Pretransfusion marrow morphology in DiGuglielmo's syndrome showing erythroid hyperplasia with abnormal mitoses (A) and bizarre multinucleated erythroid forms (B, C, D). Material shown was obtained from patients P.S. (A), D.S. (B) and W.H. (C, D).

tient D.S. The peripheral blood smear in each case showed poikilocytosis, anisocytosis, hypochromia and occasional nucleated erythroid forms.

Prior to transfusion, erythropoietin excretion was increased in the four subjects studied to between 16 and 98 standard B units per day (normal 2–6 units per day). Total erythropoiesis was two to five times greater than normal, as evidenced by the plasma iron turnover and the increased cellularity of the erythroid marrow. That this erythropoiesis was largely ineffective was indicated by the low reticulocyte index in the face of marked erythroid hyperplasia and by the low erythrocyte iron turnover.

Following the exchange transfusions there was a prompt and striking reduction in erythropoietin excretion in each instance (Fig. 2). The reduced erythropoietin stimulus was associated with a change in the marrow E:G ratio and a decrease in plasma iron turnover, erythrocyte iron turnover and reticuloocyte index (Table 1). While the cellular composition of the marrow changed
Fig. 2.—The relationship of daily erythropoietin excretion to hematocrit and corrected reticulocyte count in four patients with DiGuglielmo's syndrome subjected to hypertransfusion. Each arrow represents the time of transfusion of 1 to 4 units of compatible packed RBC.

quantitatively, a number of qualitative abnormalities persisted. Multinucleated giant erythroid forms (Fig. 1) uniformly disappeared from the marrow with transfusion, although megaloblastic erythroid precursors and both abnormal and ringed sideroblasts persisted. Giant cells with megaloblastic features were also present in the granulocytic series.
### Table 1.—Hematologic and Erythrokinetic Data in Patients with DiGuglielmo's Syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Date of Study</th>
<th>Hematocrit Per Cent</th>
<th>Retic. Index</th>
<th>Plasma Iron Turnover*</th>
<th>Per Cent Utilization</th>
<th>Erythrocyte Iron Turnover*</th>
<th>ESF Excretion †</th>
<th>Marrow E/C Ratio</th>
</tr>
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<tr>
<td>Normal</td>
<td></td>
<td></td>
<td>(1.0 ± 0.5)</td>
<td>(0.70 ± 0.15)</td>
<td>(70–90%)</td>
<td>(0.56 ± 0.15)</td>
<td>(3.9 ± 1.9)</td>
<td>(1:3–4)</td>
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<tr>
<td>C.K. DiGuglielmo's</td>
<td>8–18–61</td>
<td>26.5</td>
<td>3.8</td>
<td>3.53</td>
<td>20</td>
<td>0.71</td>
<td>(3.9 ± 1.9)</td>
<td>3.2:1</td>
<td></td>
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<tr>
<td></td>
<td>8–30–61</td>
<td>44.5</td>
<td>1.8</td>
<td>0.68</td>
<td>7</td>
<td>0.05</td>
<td></td>
<td></td>
<td>1.2:6</td>
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<tr>
<td>P.S. DiGuglielmo's</td>
<td>11–16–65</td>
<td>25.0</td>
<td>0.4</td>
<td>4.30</td>
<td>8</td>
<td>0.34</td>
<td>16.4</td>
<td>1:1.3</td>
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<tr>
<td></td>
<td>11–24–65</td>
<td>39.5</td>
<td>0.1</td>
<td>1.99</td>
<td>&lt;2</td>
<td>0.04</td>
<td>2.6</td>
<td>1:5.4</td>
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<td>D.S. DiGuglielmo's</td>
<td>7–7–66</td>
<td>21.5</td>
<td>0.6</td>
<td>1.60</td>
<td>21</td>
<td>0.34</td>
<td>98.0</td>
<td>1:1.1</td>
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<tr>
<td></td>
<td>7–25–66</td>
<td>44.3</td>
<td>0.0</td>
<td>0.35</td>
<td>&lt;1</td>
<td>0.03</td>
<td>6.2</td>
<td>1:18.1</td>
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<td>W.H. DiGuglielmo's</td>
<td>4–17–67</td>
<td>22.5</td>
<td>3.1</td>
<td>2.65</td>
<td>22</td>
<td>0.58</td>
<td>60.3</td>
<td>2.8:1</td>
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<tr>
<td></td>
<td>5–13–67</td>
<td>45.1</td>
<td>1.9</td>
<td>0.51</td>
<td>5</td>
<td>0.03</td>
<td>4.0</td>
<td>1:6.4</td>
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<tr>
<td>L.R. Lymphoma</td>
<td>4–11–66</td>
<td>32.8</td>
<td>1.2</td>
<td>1.73</td>
<td></td>
<td>21.7</td>
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<tr>
<td></td>
<td>4–13–66</td>
<td>37.5</td>
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<td>4–15–66</td>
<td>42.6</td>
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<tr>
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<td>4–19–66</td>
<td>42.5</td>
<td>0.5</td>
<td>0.68</td>
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* As mg. of iron/100 ml. whole blood/day.
† ESF (erythropoietin) excretion as standard B units/day.
The concept of neoplastic growth of marrow cells was extended to red cell precursors first by Copelli\(^2\) and then by DiGuglielmo. Terms such as “inappropriate,”\(^3\) “leukemic,”\(^2\) “neoplastic,”\(^4\) “autonomous”\(^5\) and “self-perpetuating”\(^6\) have been used to describe the bizarre erythroid proliferation seen in this disorder. In time, however, “DiGuglielmo’s syndrome” has become less clearly definable and is now generally used to designate the abnormal proliferation in both that group of patients going on to frank leukemia and in chronic sideroblastic or refractory anemias.\(^1,6,17\) Such broad usage underlines the lack of specific criteria by which these various disorders may be differentiated. The five patients described here fit into the spectrum of DiGuglielmo’s syndrome. All were characterized by morphologically abnormal marrow erythroid hyperplasia; a defect in heme synthesis was implicated in four patients by the presence of abnormal sideroblasts and by the discrepancy between total and effective erythropoiesis. In one patient, a specific acquired disorder in alpha chain synthesis (hemoglobin H) was demonstrated. Other reports in such patients describe underlying chromosomal abnormalities; these were not looked for in our patients.

The subjects reported here were transfused to a normal hematocrit in order to test the responsiveness in the erythropoietin/marrow relationship to increased oxygen supply. In each instance, transfusions resulted in decreased erythropoietin production and reduced cellularity of the erythroid marrow, consistent with previous findings. Review of the literature indicates several other instances in which the same effect was probably observed adventitiously.\(^1,3,18,19\) It is further apparent from the erythropoietin levels that the marrow hyperplasia was consistent with an erythroid marrow stimulated by erythropoietin and with an adequate iron supply. Thus the erythroid hyperplasia of DiGuglielmo’s syndrome is clearly under physiologic control. The abnormal erythroid maturation leads to ineffective red cell production and results in anemia; this, in turn, stimulates erythropoietin production and, thus, marrow erythroid hyperplasia. In terms of the regulation of erythroid proliferation, DiGuglielmo’s syndrome seems no different than cases of B12 or folate deficiency in which ineffective erythropoiesis with occasional abnormal sideroblasts are observed. Here, too, hyperplasia recedes with transfusion,\(^20\) although megaloblastic abnormalities may persist and without specific therapy there is no improvement in granulocytopenia or thrombocytopenia following the recession of the marked red cell hyperplasia.

The conclusion to be drawn from these studies is that the hyperproliferative picture in DiGuglielmo’s syndrome is not associated with abnormalities in the erythropoietin regulatory mechanism. While most of the bizarre erythroid proliferation and abnormalities of heme synthesis could be suppressed with transfusion, qualitative abnormalities of erythropoiesis persisted, as did defects in granulocyte maturation. These findings are consistent with a lesion imposed on hematopoiesis at a primitive cell level prior to differentiation along specific cell lines. The fact that a number of these patients develop typical acute granulocytic leukemia, as was clearly the case in one of our subjects, would
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indicate that at least this cell line is capable of unregulated proliferation. The concept that these cells may arise from an abnormal clone is supported by the finding of aneuploid cell lines on chromosome analysis of the marrow in patients with DiGuglielmo's syndrome.\(^2\)\(^{21}\)\(^{22}\) Dreyfus\(^23\)\(^{24}\) and Dameshek\(^16\) have previously postulated that the DiGuglielmo syndrome is a disorder, primarily of the marrow stem cell, which eventuates in acute granulocytic leukemia.

The studies reported here and the observations of others do not preclude the possible existence of leukemic erythroid proliferation. The results do suggest, however, that it is more meaningful to define the abnormalities of the erythroid marrow in the terms of disordered maturation or proliferation so that further study may be directed specifically toward the type of defect present.

REFERENCES

20. Mason, J. D., and Leavell, B. S.: The effect of transfusions of erythrocytes on un-


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