Diamond-Blackfan Syndrome: Evidence Against Cell-mediated Erythropoietic Suppression

By Melvin H. Freedman and E. Fred Saunders

The profound anemia of Diamond-Blackfan syndrome (DBS) is due to marrow red cell failure, but the pathogenesis is not understood. Studies by others indicated cell-mediated erythropoietic suppression in this condition. To explore this mechanism further, Ficoll-Hypaque-separated peripheral blood lymphocytes (PBL) from four anemic untreated patients with DBS, or from normals were cocultured with control marrow in vitro and the growth of erythropoietin-responsive stem cell colonies (CFU-E) was determined. CFU-E numbers obtained from cultures with added normal PBL were not significantly different from the number without PBL. Similarly, CFU-E from cultures with added DBS PBL were not significantly different from the number without PBL (215 versus 220, 229 versus 220 and 84 versus 60, 74 versus 94/10^5 cells, respectively). Mixing marrows from a control and one DBS patient in ratios of 2:1, 1:1, or 1:2 prior to culture failed to disclose a decrease of colony growth. We could not show cellular inhibition of erythropoiesis in these patients with DBS. The mechanism of anemia in this disorder remains an open question.

DIAMOND-BLACKFAN SYNDROME (DBS, congenital hypoplastic anemia, “erythrogenesis imperfecta”) is an example of profound marrow red cell failure. Within the first few months of life a progressive anemia develops characterized by absent reticulocytosis and reduced or absent marrow erythroid elements with preservation of other cell lines. Erythropoietin-responsive marrow stem cells have been found in untreated patients but the numbers appear to be low.1 Although it is felt to be a congenital disorder,2 the pathogenesis of the anemia is not understood.

In contrast to adults who develop acquired red cell aplasia secondary to humoral antibodies against erythropoiesis,3,4 serum inhibitory factors are lacking in DBS.5,6 Studies by others, however, indicated that peripheral blood lymphocytes (PBL) from patients with DBS suppress erythroid stem cell colony growth (CFU-E) from normal marrow in vitro.7 To explore these findings further, we cocultured whole marrow or PBL of patients with control marrow to assess any effect on CFU-E growth and proliferation.

MATERIALS AND METHODS

Patients

Four children were studied (Table 1). All fit the classic clinical and hematologic description of DBS.1 At the time of diagnosis, marrow aspirates from all showed normal cellularity with an isolated reduction of the red cell series in which erythroid elements never exceeded 4% of the differential count. Normoblasts beyond the basophilic stage were not present. At the time of the study, all were anemic and were receiving regular transfusions, and none were on corticosteroids.
Table 1. Patients With Diamond-Blackfan Syndrome

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/ Age</th>
<th>Hb (g/dl)</th>
<th>WBC (x 10^5)</th>
<th>Platelets (x 10^5)</th>
<th>Myeloid (%)</th>
<th>Lymphoid (%)</th>
<th>Promonoblasts (%)</th>
<th>Basophilic normoblasts (%)</th>
<th>Orthochromatic normoblasts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 F/5 yr</td>
<td>7.7</td>
<td>6.1</td>
<td>46</td>
<td>495</td>
<td>64</td>
<td>22</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 F/3 yr</td>
<td>7.1</td>
<td>0.0</td>
<td>7.2</td>
<td>50</td>
<td>40</td>
<td>56</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3 F/2 mo</td>
<td>6.1</td>
<td>0.1</td>
<td>10.3</td>
<td>20</td>
<td>41</td>
<td>52</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4 M/4 yr</td>
<td>8.2</td>
<td>0.0</td>
<td>5.5</td>
<td>42</td>
<td>62</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Patient 1 had previously responded to therapeutic doses of prednisone (2 mg/kg/day) as reflected by an increase of marrow erythroid elements, a rise in reticulocytes and hemoglobin level, and the loss of a transfusion requirement. Corticosteroids were stopped because of growth failure, and the anemia and transfusion requirement recurred. Patient 2 did not respond to prednisone during a 6-wk trial and was felt to be “corticosteroid resistant.” Patient 3 was newly diagnosed at the time of study; she subsequently responded to prednisone. Patient 4 originally responded to prednisone, but it was stopped because of growth failure. He was placed on a monthly transfusion program for 1 yr; subsequently there was no response to a second trial of prednisone.

Controls

PBL were obtained from 18 hematologically normal volunteers. Control marrow was obtained from children with normal erythropoiesis undergoing medical investigation for nonhematologic disorders. This study was approved by the Human Experimentation Committee of the Hospital for Sick Children.

Methods

Marrow culture for assay of CFU-E was performed as previously described. Briefly, marrow cells suspended in nutrient medium were exposed to 1.0 U/ml human erythropoietin and then immobilized in plasma clots (total volume 0.1 ml). After incubation for 7 days at 37°C in air with 5% CO₂ the clots were fixed on slides, stained with benzidine to detect heme, and examined microscopically. An erythroid colony, representing the proliferation of one progenitor CFU-E, was defined as a clump of eight or more nucleated cells showing a positive brown-orange benzidine reaction. Total number of colonies per clot was counted, quadruplicate studies averaged, and results expressed as colonies/10⁵ nucleated cells plated.

Freshly obtained PBL from patients and normals were separated from heparinized whole blood by a Ficoll-Hypaque density gradient. 1 x 10⁵ nucleated marrow cells were mixed with 10⁴, 5 x 10⁴, or 10⁵ PBL prior to plating. A control marrow and marrow from patient 3 were cultured separately and cocultured in ratios of 2:1, 1:1, and 1:2 (total cell number/culture, 1 x 10⁵). Predicted colony numbers for each coculture study were calculated and compared to actual numbers.

Table 2. Coculture Study of Control and Diamond-Blackfan Syndrome Marrow

<table>
<thead>
<tr>
<th>Marrow Source</th>
<th>CFU-E/10⁵ Nucleated Marrow Cells Plated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Result</td>
</tr>
<tr>
<td>Control</td>
<td>156</td>
</tr>
<tr>
<td>DBS</td>
<td>6</td>
</tr>
<tr>
<td>DBS:control 1:1</td>
<td>84</td>
</tr>
<tr>
<td>2:1</td>
<td>56</td>
</tr>
<tr>
<td>1:2</td>
<td>105</td>
</tr>
</tbody>
</table>
Table 3. Effect of Diamond-Blackfan Syndrome Peripheral Blood Lymphocytes on CFU-E Numbers From Control Marrow

<table>
<thead>
<tr>
<th>Patient</th>
<th>No PBL</th>
<th>10⁴ PBL</th>
<th>5 x 10⁴ PBL</th>
<th>10⁵ PBL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>220</td>
<td></td>
<td></td>
<td>215</td>
</tr>
<tr>
<td>2</td>
<td>220</td>
<td></td>
<td></td>
<td>229</td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>74</td>
<td>67</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>94</td>
<td>94</td>
<td>99</td>
<td>74</td>
</tr>
</tbody>
</table>

RESULTS

Results of combining marrow from patient 3 and control marrow are shown in Table 2. At all ratios of DBS:control marrow the actual and predicted values were almost identical, and no decrease in CFU-E was seen.

The effect of PBL from the four patients on CFU-E growth is shown in Table 3. There was no significant decrease in CFU-E at any concentration of PBL tested. Similarly, in 18 studies mean CFU-E numbers from control marrow with and without normal PBL were not significantly different (215/10⁵ ± SD 83, SE 20 versus 202/10⁵ ± SD 72, SE 17; p > 0.05).

DISCUSSION

In the cocultures of PBL from our four patients and marrows from controls we were unable to show any inhibition of erythroid colony growth in vitro. No differences were detected between studies of patients “responsive” or “resistant” to prednisone therapy. Mixing DBS and control marrow also failed to disclose a decrease of colonies.

The anemia in DBS is due to failure of red cell production, but the mechanism has not been clarified. Because of the increased incidence of developmental anomalies in this condition and its onset in early life as well as the evidence indicating genetic factors, the pathogenesis has been regarded as arising from an intrinsic erythroid stem cell abnormality. However, with the documentation of acquired serum erythropoietic inhibitors in adults with red cell aplasia, speculation arose regarding their role in the childhood condition. It is now agreed by most workers in the field that there are no serum factors suppressing red cell production in DBS.

Cellular inhibition of erythropoiesis was considered another possibility. Evidence has been presented that PBL from patients with acquired aplastic anemia suppress growth of erythroid stem cells (CFU-E) from normal marrow. The same mechanism for anemia was proposed in DBS after finding that PBL from six patients suppressed CFU-E growth by normal human marrow.

We were unable to confirm the presence of a cellular inhibitor of erythropoiesis in our patients with DBS. Possible explanations for differences in results between our data and those of others are variability in methodology from one laboratory to another and a different patient population. Patients with DBS differ clinically in their response to prednisone: some patients have an erythropoietic response with less than physiologic doses of prednisone, whereas others need doses as high as 2 mg/kg/day. Patients with DBS may fail to respond to
corticosteroids, and others respond once but not a second time. Spontaneous remissions can occur after many years of transfusions, and some patients remain in remission after corticosteroid therapy is stopped.2

Our four patients represent a cross section of DBS in terms of age, response to prednisone, and amount of blood transfusions received. Since we did not find cellular-mediated suppression of erythroid colony growth in any patient, and because serum inhibitors could not be shown in these patients previously,1,6 we conclude that an immunologic cause for the suppressed erythropoiesis in DBS is unlikely. The problem is more likely to be intrinsic to the erythroid stem cell. This could be either a quantitative deficiency or a decreased responsiveness to erythropoietin. Diminished numbers of marrow CFU-E and of peripheral blood burst-forming erythroid progenitors (BFU-E) in these patients would support this hypothesis. The pathogenesis of the erythropoietic failure in DBS remains an open question.

ACKNOWLEDGMENT
We thank Wilma Vanek and Tom Grunberger for expert technical assistance. Human erythropoietin was supplied by the National Heart, Lung and Blood Institute, Bethesda, Md.

REFERENCES
Diamond-Blackfan syndrome: evidence against cell-mediated erythropoietic suppression

MH Freedman and EF Saunders