CONCISE REPORT

Immune Suppression of Erythropoiesis in Transient Erythroblastopenia of Childhood

By Harold M. Koenig, Alton L. Lightsey, Dennis P. Nelson, and Lewis K. Diamond

Serum and IgG from four children with transient erythroblastopenia of childhood (TEC) was tested to see what effect it would have on development of erythroid colonies from bone marrow mononuclear cells. Serum and IgG specimens obtained at the time of diagnosis uniformly suppressed erythroid colony development from CFU-E. Washed bone marrow mononuclear cells from a child with TEC failed to grow in the presence of his own serum, but grew normally in the presence of isologous serum. Serum specimens obtained from patients after recovery from TEC had no effect on erythroid colony development.

The anemia of TEC appears to be due to transient immune suppression of erythroid colony development.

Transient erythroblastopenia of childhood (TEC) and congenital hypoplastic anemia (CHA) are forms of pure red cell aplasia that occur in childhood. CHA usually presents in early infancy, and most patients will be transfusion-independent when treated with intermittent corticosteroid therapy.1 TEC occurs over a wide age range in previously hematologically normal children and spontaneously remits within a few months with only supportive therapy.2

The pathogenesis of CHA and TEC is unknown. Humoral inhibitors of erythropoiesis do not occur in CHA.3 Cell-mediated inhibition of erythropoiesis was at one time thought to be the etiology of CHA,4 but subsequent studies have failed to confirm this finding.5

To determine if immune suppression of erythropoiesis occurs in TEC, we tested the serum, or IgG, from children with TEC to discover what effect it had on erythroid colony development in an erythroid colony culture system. In this culture system, bone marrow mononuclear cells in the presence of erythropoietin develop into colonies of erythroid cells after several days of incubation. Each erythroid colony represents the differentiated progeny of a single erythroid progenitor cell. This culture system thus allows the study of the effects test materials have on the early events in erythropoiesis.6

CASE REPORTS

Patient 1

A 4½-yr-old previously healthy female was admitted to the Naval Regional Medical Center, San Diego, with a 2-wk history of appearing pale. Admission laboratory data included: hemoglobin (Hb), 5.7...
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g/dl; MCV, 77 fl; and reticulocyte count, 0.6%. Hemoglobin F (HbF) level was 6%, and i-antigen was present on the red blood cell (RBC) membrane. Direct and indirect Coomb's tests were negative. Bone marrow examination revealed a slight increase in numbers of early erythroid precursors. No treatment was administered. Ten days after presentation, the child's reticulocyte count was 12%, and 50 days after admission her Hb was 12 g/dl. She remained hematologically normal for 12 mo, after which she was lost to follow-up.

Patient 2

A 12-mo-old previously healthy female was noted to be pale at the time of administration of a routine immunization. She was admitted to the Naval Regional Medical Center, San Diego, with Hb, 5.9 g/dl; MCV, 77 fl; and reticulocyte count 0.1%. HbF level was 4.8%, and i-antigen was present on the RBC membrane. Direct and indirect Coomb's tests were negative. Bone marrow erythroid precursors were markedly decreased. Her Hb fell to 4.4 g/dl over the next 7 days, so she was transfused with 100 ml of packed red cells and started on prednisone 2 mg/kg/day. Nineteen days later, her reticulocyte count was 8.1%. Over the next 4 wk her Hb rose to 12.1 g/dl. She has remained hematologically normal for 3 yr.

Patient 3

A 22-mo-old male was admitted to San Diego Children’s Hospital with a 2-wk history of lethargy. Admission laboratory data included: Hb, 7.2 g/dl; MCV, 80 fl; and reticulocyte count, 0.4%. HbF level was 3.6%; direct and indirect Coomb's tests were negative. Bone marrow examination revealed a marked decrease of erythroid precursors. The child was transfused to an Hb of 12.5 g/dl. He has been followed for 1 yr without recurrence of his anemia.

Patient 4

A 4½-yr-old female was admitted to San Diego Children’s Hospital with a 3-wk history of progressive fatigue and pallor. Admission laboratory data included: Hb, 5.7 g/dl; MCV, 90 fl; reticulocyte count, 0.6%. Direct and indirect Coomb's tests were negative. Bone marrow examination revealed a marked decrease in erythroid precursors. Over the next 4 mo the child’s reticulocyte count did not rise above 0.2%, and 4 RBC transfusions were required to maintain her Hb above 4 g/dl. After the fourth transfusion, bone marrow examination was repeated and again showed a marked decrease in erythroid precursors. HbF level at this time was 4.1 g/dl. Prednisone was started at 2 mg/kg/day, and no further transfusion were required. The child's reticulocyte count did not exceed 1% until 6 wk after starting prednisone. Eight months after presentation, the child's Hb rose to 11.8 g/dl, and prednisone was discontinued. Subsequently, she has remained hematologically normal, with no further therapy, for 4 mo.

MATERIALS AND METHODS

Serum and IgG Preparation

Serum was stored at -70°C until the time of preparation of IgG or testing. IgG was extracted from serum by ammonium sulfate precipitation and DE-52 chromatography. IgG purity was confirmed by immunoelectrophoresis. Immunoglobulin-G was diluted to a concentration of 120 μg/ml in alpha-medium (Flow Labs, Inglewood, Calif.). All specimens were sterilized prior to testing by passage through a 0.22-μ Millipore filter.

Erythroid Colony Cultures

Bone marrow was collected in sterile preservative-free heparin from children with acute leukemia who were receiving no therapy and were undergoing routine diagnostic bone marrow examination. (This procedure was approved by our hospital’s Human Experimentation Committee. Parents' consent was obtained in all cases.) The buffy coat cells were washed twice with alpha-medium, and the adherent fraction of cells was removed by a 1-hr incubation of the buffy coat cells in tissue culture ware. The nonadherent mononuclear cells were cultured at a concentration of 10^6 cells/ml in 0.8% methyl cellulose (Dow Chemical Co., Midland, Mich.) with 2.5 IU/ml of sheep erythropoietin (Connaught, Step IV, Willowdale, Ontario, Canada) according to the method of Iscove. Cultures contained 1.1 ml of culture material plus 0.1 ml of test material and were incubated at 37°C, 95% relative humidity in 5% CO₂.
Erythroid Colony Scoring

Cultures were scored for colony development by counting at 65X magnification with a blue filter under an inverted microscope after 8, 12, and 18 days of incubation. Colonies were counted as erythroid only if they contained hemoglobin. Colonies containing 1 or 2 clusters (CFU-E) were scored at 8 days of culture, colonies with 3–8 clusters (mature BFU-E) were scored at 12 days of culture, and colonies with greater than 16 clusters (primitive BFU-E) were scored at 18 days of culture.

RESULTS

The effect of IgG in a concentration of 100 μg/ml isolated from serum of patients 1, 2, and 3 at the time of diagnosis and a concentration of 8% heat-inactivated serum from patient 4 at the time of diagnosis on erythroid colony development is shown in Table 1. The mean number of colonies that developed from CFU-E were significantly lower \((p < 0.01)\) in all of the cultures containing patient’s IgG or serum than were the number of colonies that developed in control cultures. The number of colonies that developed from mature BFU-E was significantly lower \((p < 0.05)\) in 3 of 4 specimens tested when compared to controls. The number of erythroid colonies that developed from primitive BFU-E was significantly lower than controls only in cultures containing serum from patient 4.

After patient 4 received her fourth blood transfusion, a second bone marrow aspiration was done prior to starting corticosteroid therapy. Mononuclear cells from this bone marrow were cultured simultaneously with bone marrow from a control patient in serum and cell-mixing experiments, shown in Table 2. Erythroid colonies developed from both the patient’s cells and control cells in the presence of added control serum or alpha-medium. However, when the patient’s serum was added to either her own cells or the control cells, erythroid colony development from CFU-E and mature and primitive BFU-E was nearly totally prevented. Subsequently, IgG was isolated from the serum of patient 4 and shown to suppress erythroid colony development. Serum samples obtained from the four children after they had become hematologically normal did not suppress erythroid colony development.

DISCUSSION

Pure red cell aplasia in childhood has been associated with infections, renal failure, severe malnutrition, marrow depressant drugs, thymic tumors, and immune hemolysis. CHA and TEC are forms of pure red cell aplasia that are unassociated with underlying disorders and of unknown etiologies. The conditions differ in that

<table>
<thead>
<tr>
<th>Culture Contents</th>
<th>CFU-E</th>
<th>Mature BFU-E</th>
<th>Primitive BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>81</td>
<td>21</td>
<td>8</td>
</tr>
<tr>
<td>Normal IgG</td>
<td>85</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>Patient 1, IgG</td>
<td>65</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>Patient 2, IgG</td>
<td>66</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Patient 3, IgG</td>
<td>60</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Patient 4, Serum</td>
<td>43</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

*Values represent mean of duplicate samples.
Table 2. Effect of Serum From a Child With Transient Erythroblastopenia on Erythroid Colony Production From Her Own and Control Bone Marrow Cells

<table>
<thead>
<tr>
<th>Bone Marrow Source</th>
<th>Test Material Added</th>
<th>Erythroid Colonies Derived From*</th>
<th>CFU-E</th>
<th>Mature BFU-E</th>
<th>Primitive BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>None</td>
<td>178</td>
<td>46</td>
<td>37</td>
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</tr>
<tr>
<td>Control</td>
<td>Control serum</td>
<td>175</td>
<td>46</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Patient serum</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>None</td>
<td>133</td>
<td>34</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>Control serum</td>
<td>139</td>
<td>33</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>Patient serum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Values represent mean of quadruplicate samples.

at the time of diagnosis children with TEC have an anemia characterized by red blood cells of near normal size, fetal hemoglobin content, i-antigen score, and enzyme activity for their age. In contrast, children with CHA have macrocytic red blood cells that contain elevated levels of fetal hemoglobin, increased activity of certain enzymes, and elevated i-antigen scores. These findings, characteristic of fetal erythropoiesis, are present in periods of spontaneous or corticosteroid-induced remission and become more pronounced during periods of relapse. Children in the recovery phase of TEC may transiently develop macrocytic red blood cells with elevated levels of hemoglobin F, i-antigen scores, and enzyme activities. The development of red blood cells with fetal characteristics is thought to be a nonspecific response seen during recovery from severe anemia. The overall prognosis for the two conditions differs greatly in that children with TEC recover quickly and spontaneously, while CHA is a disorder usually requiring life-long intermittent corticosteroid therapy or transfusion support.

Recently, studies have been done to try to determine the pathogenesis of CHA. These studies have shown that normal numbers of erythroid colonies can be grown from bone marrow cells of children with congenital hypoplastic anemia who are in spontaneous or corticosteroid-induced remission. However, erythroid colony formation from bone marrow mononuclear cells is decreased in patients in relapse. One study has proposed that patients with congenital hypoplastic anemia have primitive BFU-E that will not respond to erythropoietin except in the presence of corticosteroids. Inhibitors of erythropoiesis have not been found in the plasma of patients with congenital hypoplastic anemia. Lymphocyte-mediated suppression of erythropoiesis was suggested as the etiology of congenital hypoplastic anemia by one group of investigators, but subsequent studies have been unable to confirm these findings.

In this study, we have shown that normal numbers of erythroid colonies developed from the bone marrow mononuclear cells of a child with TEC when her cells were cultured in a system free of her own plasma. We have also demonstrated that IgG isolated from serum from children with TEC will suppress erythroid colony development in culture and that this suppressive activity disappears after hematologic recovery occurs.

Erythroid colony formation from CFU-E, which have a low erythropoietin growth requirement, was suppressed to a much greater degree than was erythroid colony development from primitive BFU-E, which have a high erythropoietin
requirement, in cultures containing test serum or IgG. These findings suggest that
the anemia of transient erythroblastopenia of childhood is caused by an immune
mechanism that directly suppresses erythroid progenitor cell differentiation and not
by blocking erythropoietin.

ACKNOWLEDGMENT

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and Diana Seaward for her technical assistance.

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