Autosomal Dominant Familial Erythrocytosis Due to Autonomous Erythropoietin Production

By Clark W. Distelhorst, Douglas S. Wagner, Eugene Goldwasser, and John W. Adamson

A family is described in which four members spanning three consecutive generations have erythrocytosis associated with a normal hemoglobin oxygen affinity. When bone marrow from one affected family member was cultured in vitro, erythroid colonies formed only when erythropoietin was added to the culture. Serum erythropoietin, measured by radioimmunoassay, was significantly elevated above normal in each of the affected family members. Bioassayable erythropoietin was detected in the urine of two of the three affected family members. In two of the affected family members, erythropoietin was measured in serum by radioimmunoassay and in urine by bioassay and was present at a level of 1000 ml of blood.

Case 1

A 13-yr-old male had been in excellent health until November 1979, when he developed fatigue, anorexia, and headache. With the exception of plethora, his physical examination was normal. The spleen was not palpable. Initial laboratory data included hemoglobin 20.2 g/dl, hematocrit 60%, white cell count 6700/dl, with a normal differential, platelet count 162,000/λ and reticulocyte count 1.0%. Red cell indices and morphology were normal. Arterial blood gases and oxygen saturation, leukocyte alkaline phosphatase, serum vitamin B<sub>12</sub>, and unsaturated vitamin B<sub>12</sub> binding capacity (UBBC) were normal. The 9-1Cr-labeled red cell volume was 3200 ml with a predicted normal of 2214 ml. Hemoglobin electrophoresis was normal. Chest x-ray, intravenous pyelogram, and computerized tomography of the head and abdomen were normal. Bone marrow examination revealed mild erythroid hyperplasia. Isovolemic phlebotomies of 1000 ml of blood were performed in November 1979 and February 1980, with symptomatic relief.

Case 2

The 9-yr-old brother of case 1 was asymptomatic with a normal physical examination. His hemoglobin was 17.5 g/dl, hematocrit 53%, and white cell count 5200/dl, with a normal differential. In February 1980, serial urine and serum samples were collected for erythropoietin studies before and after an isovolemic phlebotomy of 500 ml of blood.

Case 3

The 30-yr-old mother of the two siblings described above was asymptomatic and in good health except for menorrhagia. She received supplemental oral iron during a pregnancy 5 yr earlier. Her hemoglobin was 14.6 g/dl, hematocrit 45%, and white cell count 7400/dl. Her serum iron was 50 μg/dl and total iron binding capacity was 410 μg/dl. After treatment with ferrous sulfate for 6 mo, her serum iron and iron binding capacity were unchanged, but her hemoglobin was 15.9 g/dl and her hematocrit 50%. Hemoglobin electrophoresis was normal.

Case 4

The 57-yr-old maternal grandfather of cases 1 and 2 had recurrent malaria in the past. In 1975 he developed lethargy and plethora and was found to have a hemoglobin of 17.5 g/dl and hematocrit of 52%. At that time his physical examination was normal and his spleen was not palpable. He received symptomatic relief from periodic phlebotomies. He was not evaluated further. In November 1979, his hemoglobin was 18.2 g/dl, hematocrit 56%, white cell count 11,300/dl, and platelet count 319,000/λ. His serum vitamin B<sub>12</sub> level and UBBC were normal.

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MATERIALS AND METHODS

Red Cell Function Studies

Whole blood oxygen dissociation curves were constructed by the Ohio State University Special Hematology Laboratory by a previously published method. The hemoglobin oxygen affinity ($P_50$) was also calculated from the venous oxygen tension and oxygen saturation with the formula of Lichtman et al. Erythropoietin Studies

Erythropoietin was measured on aliquots of pooled daily urine collections by means of a posthypoxic polycythemic mouse assay system as described previously. A 24-hr urine collection, initially frozen with each voiding, was thawed, mixed, and one-fourth removed for concentration. The concentrate was freed of insoluble material and injected on 2 consecutive days into suitably prepared assay animals. Radioactive iron incorporation was used to monitor erythropoietic stimulation and the quantitation of erythropoietin excretion was achieved by comparing results obtained with test material to results with simultaneously assayed erythropoietin standards. Serum erythropoietin was measured by radioimmunoassay by a previously published method. Urine and serum were collected for 2 days prior to and for 4 days following a single isovolemic phlebotomy designed to reduce the red cell mass by 20%. The blood removed was replaced quantitatively with plasma protein preparation (Plasmanate, Cutter Laboratories, Berkeley, Calif.). Urine and serum samples were stored at $-20\,^\circ\text{C}$ until assayed for erythropoietin.

Erythroid Culture

Peripheral blood and bone marrow obtained from case 1 were shipped in preservative-free heparin at ambient temperature to Seattle, Wash., and in vitro cultures were established within 12 hr after the marrow was obtained for erythroid colony growth. Ficoll-Hypaque separated peripheral blood mononuclear cells and buffy-coat-enriched marrow cells were cultured by previously described methods in the presence and absence of added erythropoietin (Step III sheep plasma erythropoietin; 12.4 U/mg; Connaught Laboratories, Willowdale, Ontario). Colonies derived from erythroid colony-forming cells (CFU-E) appearing at 6–8 days of culture contained $8–64$ hemoglobinized cells and were enumerated with an inverted tissue culture microscope. Erythroid bursts, derived from burst-forming units (BFU-E) and containing up to $10^3$ cells were enumerated on day 12 of culture.

The studies described here were approved by the Human Experimentation Committee of The Children’s Hospital Research Foundation. Informed consent was obtained prior to phlebotomies and marrow aspiration.

Fig. 2. Daily excretion and serum levels of erythropoietin (ESF) before and after phlebotomy in two brothers with erythrocytosis. Arrow indicates day on which phlebotomy was performed. Normal serum ESF is $14.9 \pm 4.2\,\text{mU/mL}$.
RESULTS

Red-Cell Function Studies

The P\(_{50}\) derived from the whole-blood oxygen dissociation curve of case 1 was 23.9 mm Hg (normal 21.5–25.0). Hill’s n was 2.46 (normal 2.40–2.80). The P\(_{50}\) calculated from venous blood gas measurements was 27.5 mm Hg (normal 26 ± 1.4, mean ± SD).

Erythropoietin Studies

Baseline bioassayable erythropoietin was detected in the urine of cases 1 and 3, but not in case 2 (Fig. 2 and Table 1). The absence of detectable erythropoietin in the urine of case 2 is consistent with previous observations that erythropoietin cannot be detected in urine concentrates from hematologically normal prepubertal children (J.W. Adamson, unpublished results). Immunossayable serum erythropoietin levels were elevated above normal in each of the affected family members (Fig. 2 and Table 1). Neither urinary erythropoietin excretion nor serum erythropoietin levels increased following phlebotomy (Fig. 2). A reticulocytosis did not develop in response to the reduction in hematocrit (Fig. 2), although serum iron levels were normal.

Erythroid Culture Studies

When bone marrow cells obtained from case 1 were cultured, erythroid colonies did not form in the absence of exogenous erythropoietin. When erythropoietin was added to the cultures, increasing numbers of erythroid colonies formed in cultures to which erythropoietin was added. Therefore, a stem cell defect analogous to that which occurs in polycythemia vera, was responsible for the erythrocytosis in the family described here.

In vitro marrow culture produces erythroid colony formation in the absence of exogenous erythropoietin. However, a similar pattern of in vitro erythroid colony formation has been observed in patients who do not have the full clinical and laboratory picture of polycythemia vera. Greenberg and Golde\(^5\) described two brothers with erythrocytosis who failed to meet the diagnostic criteria of the Polycythemia Vera Study Group,\(^12\) although in vitro marrow culture revealed endogenous erythroid colony formation. This observation suggested that an abnormality of erythroid-committed stem cells, analogous to the stem cell defect in polycythemia vera, was responsible for the erythrocytosis in these two siblings. Unlike these cases and polycythemia vera, Dainiak et al.\(^1\) described a case in which erythrocytosis was associated not only with abnormal erythropoietin production but also endogenous erythroid colony formation.

In order to evaluate erythropoiesis at the stem cell level in the family described here, we cultured marrow from case 1 in vitro in the presence and absence of exogenous erythropoietin. Endogenous erythroid colony formation was not observed, but normal numbers of erythroid colonies formed in cultures to which erythropoietin was added. Therefore, a stem cell defect analogous to that which occurs in polycythemia vera does not appear to be responsible for the erythrocytosis in the family described here.

<table>
<thead>
<tr>
<th>Case</th>
<th>Serum Radioimmunoassay (mU/ml)</th>
<th>Urine Bioassay (U/Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>Not detectable</td>
</tr>
<tr>
<td>3</td>
<td>66</td>
<td>1.3</td>
</tr>
<tr>
<td>4</td>
<td>38</td>
<td>Not done</td>
</tr>
<tr>
<td>Normal*</td>
<td>14.9 ± 4.2 (n = 26)</td>
<td>4.2 ± 1.3 (n = 21)†</td>
</tr>
</tbody>
</table>

*Mean ± SD.
†Control value obtained by study of adults.

DISCUSSION

Erythropoiesis is regulated by a number of factors, including tissue oxygen tension, erythropoietin production, and the pool of erythropoietin-responsive stem cells in the bone marrow. Defects at any of these levels could potentially lead to increased red cell production and erythrocytosis. We have described a family in which four members spanning three generations have erythrocytosis associated with a normal arterial oxygen saturation and normal hemoglobin oxygen affinity. In order to determine the mechanism responsible for erythrocytosis in the family, we have studied erythropoiesis at both the stem cell and humoral levels.

In vitro culture of bone marrow from patients with polycythemia vera reveals abnormal erythroid colony formation. Zanjani et al.\(^11\) demonstrated that in polycythemia vera, but not in normal individuals, in vitro marrow culture produces erythroid colony formation in the absence of exogenous erythropoietin. However, a similar pattern of in vitro erythroid colony formation has been observed in patients who do not have the full clinical and laboratory picture of polycythemia vera.
of bioassayable erythropoietin is unchanged following phlebotomy, indicating autonomous erythropoietin production independent of normal control mechanisms. Yonemitsu et al. described two siblings with erythrocytosis in whom baseline urinary and plasma bioassayable erythropoietin levels were elevated. However, the response of the erythropoietin levels to phlebotomy was not studied so it is not possible to know if erythropoietin production was autonomous. In the case of erythrocytosis described by Dainiak et al. baseline erythropoietin levels were greater than normal and increased fourfold following phlebotomy. In this case, therefore, erythropoietin production was not autonomous but possibly due to an elevation in the setpoint of the oxygen sensor, which regulates erythropoietin production.

In the affected members of our family, erythropoietin levels in serum, measured by radioimmunoassay, were significantly elevated above normal (case 1, 48 mU/ml; case 2, 56 mU/ml; case 3, 66 mU/ml; case 4, 38 mU/ml). Koeffler and Goldwasser found that 91% of patients with secondary polycythemia who were studied by the same radioimmunoassay had erythropoietin levels greater than 30 mU/ml and that 92% of patients with polycythemia vera had erythropoietin levels less than 30 mU/ml. Therefore, the serum erythropoietin levels in the affected family members described here were in the range observed in secondary polycythemias. Significantly, in cases 1 and 2, serum erythropoietin levels did not increase following phlebotomy. This finding was confirmed by measurements of the urinary excretion of bioassayable erythropoietin and the absence of a reticulocyte response to the phlebotomy. These results are similar to those previously reported by Adamson et al. for members of two families in which erythrocytosis was associated with an abnormality in erythropoietin production similar to that observed here. In those cases, and in the family described here, erythrocytosis appears to be secondary to autonomous erythropoietin production.

The erythrocytosis in the two families described by Adamson et al. appeared to be inherited in a recessive fashion. The inheritance pattern in the family reported here appears to be autosomal dominant with involvement of three consecutive generations. The failure of case 3 in this family to develop the same degree of erythrocytosis as was observed in the other affected family members may indicate variable expression of a dominant gene, but more likely is due to blood loss and iron deficiency secondary to menorrhagia. Familial erythrocytosis with dominant inheritance is generally due to mutant hemoglobins with increased oxygen affinity. Several families with erythrocytosis inherited on a dominant basis but with normal hemoglobin oxygen affinity have been reported. However, the mechanisms responsible for the erythrocytosis in these families have not been defined. Therefore, this is the first report of familial erythrocytosis inherited on a dominant basis in which the erythrocytosis appears to be caused by unregulated erythropoietin production.

REFERENCES

Autosomal Dominant familial erythrocytosis due to autonomous erythropoietin production

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