Autosomal Dominant Erythrocytosis Caused By Increased Sensitivity to Erythropoietin

By Eeva Juvonen, Eero Ikkala, Frej Fyhrrquist, and Tapani Ruutu

We describe here a family with autosomal dominant erythrocytosis. In in vitro cultures, performed using the methyl cellulose assay, the number of erythroid colonies was normal or marginally increased when a standard concentration of erythropoietin (Epo) was used, but at lower Epo concentrations, the investigated persons formed more colonies than the controls. The difference was generally greater the lower the Epo concentration became. Some erythroid colony growth was seen even in the absence of any added Epo (apart from the minute concentration found in fetal calf serum), a phenomenon not seen in the controls. This finding indicates that the erythrocytosis in this family is caused by hypersensitivity of erythroid progenitors to Epo. The serum Epo concentration was low or low normal in all of the investigated family members, which is in good accordance with hypersensitivity to Epo. The erythrocytosis has not had any obvious effect on the health or life-span of the affected individuals. Many of them have reached an advanced age, and one of the affected family members has won several Olympic gold medals and world championships in endurance sports.

Familial erythrocytosis is a heterogeneous group of hereditary conditions with an increased total red blood cell (RBC) volume. In most families, the erythrocytosis is due to an abnormal hemoglobin (Hb) molecule with increased oxygen affinity. Abnormal metabolism of 2,3-diphosphoglycerate (DPG) is another, more rare cause of hereditary erythrocytosis. In some families, erythrocytosis is caused by autonomous production of erythropoietin (Epo). A familial form of polycthemia vera has also been reported. Erythrocytosis caused by abnormal Epo sensitivity and inherited by an autosomal recessive or dominant mode has been described. In some families, the mechanism of erythrocytosis is not known. We describe here a family with autosomal dominant erythrocytosis caused by excessive sensitivity of erythroid progenitors to Epo. The propositus has been one of the best cross-country skiers in the world. Skiing with Hb values greater than 200 g/L he has won several Olympic gold medals and world championships.

FAMILY

Three members of a large family from Northern Europe with familial erythrocytosis were investigated in the present study (Fig 1). Several members of the family have previously been examined at different hospitals because of high RBC levels, but the cause of the erythrocytosis has remained unclear. No abnormalities in the Hb molecule have been found, and the oxygen dissociation curve has been normal in all investigated members of the family. To study the mode of inheritance, the Hb concentration of 97 family members was measured. Ten individuals declined to participate in the investigation. She has had erythrocytosis since childhood. She has always been in good health and has never had any symptoms related to erythrocytosis. A BM aspirate showed erythroid hyperplasia. She has been operated on for an ovarian cyst without any problems.

The cousin of the propositus, a 31-year-old male, has also had high Hb levels since childhood. He has always been in excellent health. He has won several Olympic gold medals and world championships in cross-country skiing. His Hb levels have been 200 g/L or more since childhood. The laboratory findings at the time of the present investigation are shown in Table 1. The RBC indices were within normal limits. The reticulocyte count was also normal (0.010). The total RBC volume was 4,782 mL or 59.0 mL/kg (normal, 25 to 35), and the 2,3-DPG concentration was slightly reduced (2.9 mmol/L; normal, 3.1 to 5.9). The isoelectric focusing of Hb was normal. A bone marrow (BM) aspirate showed erythroid hyperplasia. The chest x-ray and electrocardiography were normal. The spleen size measured radiologically was normal. The propositus has never smoked. A cholecystectomy for gallstones was performed without problems.

The daughter of the propositus was 23 years old at the time of the investigation. She has had erythrocytosis since childhood. She has always been in good health and has never had any symptoms related to erythrocytosis. A BM aspirate showed erythroid hyperplasia. She has been operated on for an ovarian cyst without any problems.

The propositus, a 53-year-old male, has always been in excellent health. He has won several Olympic gold medals and world championships in cross-country skiing. His Hb levels have been 200 g/L or more since childhood. The laboratory findings at the time of the present investigation are shown in Table 1. The RBC indices were within normal limits. The reticulocyte count was also normal (0.010). The total RBC volume was 4,782 mL or 59.0 mL/kg (normal, 25 to 35), and the 2,3-DPG concentration was slightly reduced (2.9 mmol/L; normal, 3.1 to 5.9). The isoelectric focusing of Hb was normal. A bone marrow (BM) aspirate showed erythroid hyperplasia. The chest x-ray and electrocardiography were normal. The spleen size measured radiologically was normal. The propositus has never smoked. A cholecystectomy for gallstones was performed without problems.

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MATERIALS AND METHODS

Erythroid progenitors from the BM and blood were cultured in a methyl cellulose assay as described previously. For the BM cultures, 2 mL of marrow was aspirated and diluted in Iscove's modified Dulbecco's medium (IMDM) containing heparin, and for the cultures of circulating progenitors, 10 to 20 mL of venous blood was collected. Mononuclear cells were isolated by Ficoll-Isoaque gradient from both types of samples. The cells were washed and resuspended in IMDM. The culture medium consisted of 0.8% methyl cellulose, 20% fetal calf serum, 1% delipidated and deionized bovine serum albumin, 10−4 mol/L 2-mercaptoethanol, 310 μg/mL fully iron-saturated human transferrin, 20% human serum.
leukocyte-conditioned medium prepared in IMDM as described by Iscove et al. and IMDM. In the BM cultures, the number of mononuclear cells was \(5 \times 10^4/mL\), and in the blood cultures \(2 \times 10^4/mL\). The growth of erythroid progenitors was stimulated with varying concentrations of recombinant Epo (Amgen, Thousand Oaks, CA). Some cultures were performed without exogenous Epo. The colonies for colony-forming unit-erythroid (CFU-E) were scored after 7 days of incubation and those for burst-forming unit-erythroid (BFU-E) after 14 days of incubation at 37°C in a fully humidified atmosphere with 5% CO₂.

The erythroid colony-stimulating activity of the plasma of the investigated persons was also tested. The culture assay consisted of 30% of human plasma, 5% phytohemagglutinin-stimulated leukocyte-conditioned medium, 5 \(\times 10^{-3}\) mol/L 2-mercaptoethanol and 0.9% methyl cellulose in IMDM, and 5 \(\times 10^4\) mononuclear cells from normal BM.

Granulocyte-macrophage and megakaryocyte progenitors were cultured as described previously.

Seven BM transplant donors served as normal controls.

The serum Epo concentration was measured by radioimmunoassay, in which recombinant Epo was used for immunization, for radiolabeling, and as a standard.

Approval was obtained from the Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki.

RESULTS

In the cultures stimulated with a standard concentration of Epo (1 U/mL) two investigated persons showed normal erythroid colony growth, while the third had increased colony formation when compared with the colony numbers of the normal controls (Table 2). At lower Epo levels, the erythroid colony formation tended to be more abundant in the investigated persons than in the normal controls at the given Epo concentration. The lower the Epo concentration was, the clearer was the difference between the investigated persons and the controls. The difference was more marked in the numbers of the CFU-E colonies than in those of the BFU-E colonies. In the assays without exogenous Epo, all the investigated persons, but none of the controls, showed a few erythroid colonies.

Figure 2 shows the numbers of the erythroid colonies formed at different Epo concentrations expressed as a percentage of the number of colonies formed at an Epo concentration of 1 U/mL. A higher proportion of erythroid progenitors from the investigated persons formed colonies at a lower suboptimal Epo concentration when compared with that of erythroid progenitors from the controls. At the lower Epo concentrations, most of the erythroid colonies of the investigated persons appeared normal, whereas in the cultures from the controls, most colonies were small and poorly hemoglobinized. The difference between the investigated persons and the controls was more marked in the numbers of the CFU-E colonies than in those of the BFU-E colonies.

In the cultures of blood BFU-E, the results were similar to those of the BM cultures (data not shown).

The granulocyte-macrophage and megakaryocyte colony formation was normal in all three investigated persons.

The serum Epo concentration of the investigated persons

Table 1. Laboratory Data of the Investigated Family Members

<table>
<thead>
<tr>
<th></th>
<th>Propositus (male)</th>
<th>Daughter</th>
<th>Cousin (male)</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/L)</td>
<td>231</td>
<td>193</td>
<td>225</td>
<td>125-160</td>
<td>135-180</td>
</tr>
<tr>
<td>Hcr (%)</td>
<td>68</td>
<td>57</td>
<td>68</td>
<td>37-47</td>
<td>40-54</td>
</tr>
<tr>
<td>RBC ((\times 10^11)/L)</td>
<td>7.74</td>
<td>6.30</td>
<td>7.68</td>
<td>4.00-5.30</td>
<td>4.50-6.10</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>88</td>
<td>91</td>
<td>89</td>
<td>80-96</td>
<td>80-96</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>30</td>
<td>31</td>
<td>29</td>
<td>27-32</td>
<td>27-32</td>
</tr>
<tr>
<td>MCHC (g/L)</td>
<td>341</td>
<td>335</td>
<td>331</td>
<td>320-360</td>
<td>320-360</td>
</tr>
<tr>
<td>WBC ((\times 10^9)/L)</td>
<td>4.6</td>
<td>7.4</td>
<td>7.1</td>
<td>4.0-10.0</td>
<td>4.0-10.0</td>
</tr>
<tr>
<td>Platelets ((\times 10^9)/L)</td>
<td>162</td>
<td>177</td>
<td>153</td>
<td>150-450</td>
<td>150-450</td>
</tr>
</tbody>
</table>

Abbreviations: Hcr, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; MCHC, MCH concentration.
Table 2. Effect of Epo Concentration on Erythroid Colony Formation

<table>
<thead>
<tr>
<th>Epo (U/mL)</th>
<th>Propositus BFU-E</th>
<th>Propositus CFU-E</th>
<th>Daughter BFU-E</th>
<th>Daughter CFU-E</th>
<th>Cousin BFU-E</th>
<th>Cousin CFU-E</th>
<th>Normal Controls BFU-E</th>
<th>Normal Controls CFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>118</td>
<td>204</td>
<td>96</td>
<td>268</td>
<td>288</td>
<td>644</td>
<td>50-250</td>
<td>90-375</td>
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<tr>
<td>0.1</td>
<td>74</td>
<td>121</td>
<td>76</td>
<td>170</td>
<td>178</td>
<td>413</td>
<td>24-154</td>
<td>27-140</td>
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<tr>
<td>0.05</td>
<td>86</td>
<td>188</td>
<td>73</td>
<td>158</td>
<td>159</td>
<td>408</td>
<td>20-95</td>
<td>18-85</td>
</tr>
<tr>
<td>0.025</td>
<td>78</td>
<td>144</td>
<td>68</td>
<td>114</td>
<td>110</td>
<td>405</td>
<td>4-47</td>
<td>0-16</td>
</tr>
<tr>
<td>0</td>
<td>15</td>
<td>8</td>
<td>12</td>
<td>5</td>
<td>10</td>
<td>92</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are the number of BFU-E and CFU-E colonies per $5 \times 10^6$ mononuclear BM cells.

was low or low normal: in the propositus 8.6 pmol/L (39 U/mL); and in the daughter and cousin less than 8 pmol/L (<36 U/mL) (normal values, 8 to 43 pmol/L or 36 to 195 U/mL). The plasma of the patients did not stimulate the in vitro colony formation of normal erythroid progenitors, which was in accordance with their low Epo concentrations.

DISCUSSION

In the present study we describe a Finnish family with erythrocytosis inherited in an autosomal dominant fashion. The mechanism of the erythrocytosis seems to be increased sensitivity to Epo. In a standard in vitro culture assay, at 1 U/mL of Epo two of the three investigated persons grew normal numbers of erythroid colonies, while the third had increased numbers of colonies. At the lower Epo concentrations, all three investigated persons showed higher numbers of colonies than the controls, and the difference was greater the lower the Epo concentration became. Even in the absence of added exogenous Epo, all three investigated persons grew a few erythroid colonies, a phenomenon not seen in any of the controls. The low or low normal serum Epo concentration in the investigated persons is in accordance with the interpretation of increased Epo sensitivity of progenitors as the cause of erythrocytosis.

The most common cause for familial erythrocytosis is an inherited Hb mutant with increased oxygen affinity leading to a leftward shift of the oxygen dissociation curve. No abnormalities in the Hb molecule were found in the present family.

Inherited abnormalities in the regulation of erythrocytosis are rare causes of familial erythrocytosis. Distelhorst et al. have described a family with autosomal dominant familial erythrocytosis caused by autonomous Epo production, and Dainiak et al. have reported a patient with primary pure erythrocytosis due to increased Epo production, possibly recessively inherited. Familial pure erythrocytosis caused by increased Epo sensitivity of restricted erythroid progenitors is also known. Prchal et al. have described two families with erythrocytosis inherited in an autosomal dominant fashion. Family 1 in their study greatly resembles the family presented here. The sensitivity of erythroid progenitors to Epo was increased, the Epo concentration in the serum of the affected family members was decreased, and also a few erythroid colonies were seen without exogenous Epo, as in the family described here.

Epo is a glycoprotein hormone that acts on committed erythroid progenitors, mature BFU-E, and CFU-E. Pluripotent stem cells and primitive BFU-E do not respond to Epo. In normal erythropoiesis, a higher concentration of Epo is needed for in vitro colony formation of BFU-E than for CFU-E, and other regulators may also have a more important regulatory role for BFU-E colony growth. Changes in the Epo sensitivity during differentiation have been thought to be due to a change in the number or affinity of the receptors. In the present family, the abnormal in vitro sensitivity to Epo was more obvious in the colony formation of CFU-E than in that of BFU-E. In this respect, it was in accordance with normal erythropoiesis. The mechanism of
the increased Epo sensitivity in this family is not known. An analysis of Epo receptors would have been most interesting, but it was not available for us.

The impact of the erythrocytosis on the health of the affected members of the present family is uncertain, but no clearly erythrocytosis-related complications have been observed. Some cardiovascular disorders at advanced age have occurred, mostly in individuals not known to have erythrocytosis. In general, the erythrocytosis in the present family seems to cause few problems and is compatible with long life, good health, and even exceptional physical fitness, as shown by the propositus. The presence of erythrocytosis in the propositus with remarkable sports achievements should not encourage the increase of RBC levels by artificial means, especially Epo, to improve physical performance. The risk of serious side effects is considerable, not to speak of ethical aspects. Life-long adaptation to high RBC levels may have protected the propositus from complications.

REFERENCES

Autosomal dominant erythrocytosis caused by increased sensitivity to erythropoietin

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