Insulin-Like Growth Factor I Plays a Role in Regulating Erythropoiesis in Patients with End-Stage Renal Disease and Erythrocytosis

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Abstract. Erythroid progenitor growth, the serum hormones that regulate erythropoiesis, and the effect of patient’s serum on the growth of normal erythroid progenitors were assessed in eight patients with end-stage renal disease (ESRD) and erythrocytosis. All patients were male and had been on maintenance dialysis, they had a hematocrit >50% and/or a red blood cell count >6 × 10¹²/L and an arterial oxygen saturation >95%. Four had acquired cystic disease of the kidney (ACDK), and four other non-ACDK patients did not have known causes of secondary erythrocytosis after appropriate investigations and long-term follow-up. The methylcellulose culture technique was used to assay the erythroid progenitor (BFU-E/CFU-E) growth. Serum erythropoietin (EPO) and insulin-like growth factor I (IGF-I) levels were measured by RIA. Paired experiments were performed to determine the effects of 10% sera from ESRD patients and control subjects on normal marrow CFU-E growth. The numbers of EPO-dependent BFU-E in marrow and/or blood of patients with ESRD and erythrocytosis were higher than those of normal controls. No EPO-independent erythroid colonies were found. Serum EPO levels were constantly normal in one patient and elevated in three patients with ACDK; for non-ACDK patients, EPO levels were normal or low in two patients and persistently increased in one, but fluctuated in the remaining one on serial assays. There was no correlation between serum EPO levels and hematocrit values. The serum IGF-I levels in patients with ESRD and erythrocytosis were significantly increased compared with normal subjects or ESRD patients with anemia. We found an inverse correlation between serum EPO and IGF-I levels. Sera from patients with ESRD and erythrocytosis exhibited a stimulating effect on normal marrow CFU-E growth. The stimulating effect of sera from patients who had a normal serum EPO level and an elevated IGF-I level could be partially blocked by anti-IGF-I. The present study suggests that IGF-I plays an important role in the regulation of erythropoiesis in patients with ESRD and erythrocytosis who did not have an increased EPO production.

Erythrocytosis may complicate the disease course of renal tumor (1), cysts (2), polycystic kidney (3), hydronephrosis (4), renal artery stenosis (5), and postrenal transplant (6,7). In most of these patients, the renal function was normal or only mildly impaired. On the contrary, anemia of varying degrees is invariably present in patients with chronic renal failure except for some patients with polycystic kidney disease (8,9). The presence of erythrocytosis in the setting of end-stage renal disease (ESRD) is an extremely rare phenomenon. There have been rare reports that described only one or two patients with ESRD and erythrocytosis of a variety of etiologies including acquired cystic disease of the kidney (ACDK) (10), secondary erythrocytosis due to hypoxia (11), polycystic kidney disease with renal artery thrombosis (12), diabetes mellitus (13), and polycythemia vera (14–16). We encountered eight patients with erythrocytosis and ESRD on maintenance dialysis: four had ACDK and the remaining four did not fit into polycythemia vera or any known mechanism of secondary erythrocytosis. We assessed the in vitro erythroid progenitor growth, serum erythropoietin (EPO), and insulin-like growth factor I (IGF-I) levels, and the effects of sera from uremic patients on the growth of normal erythroid progenitors. Our results suggest that IGF-I is an important regulator in some patients with ESRD and erythrocytosis. To the best of our knowledge, IGF-I-mediated erythrocytosis in patients with ESRD has not been described previously.

Materials and Methods

Patient Population

From 1982 to 1996, 13 patients undergoing hemodialysis were found to be associated with erythrocytosis at Chang Gung Memorial Hospital, where the Hemodialysis Center serves 850 patients for regular dialysis. Of them, five with clinical and laboratory features that fulfilled the diagnostic criteria of polycythemia vera were excluded (17); the remaining eight patients constituted the basis of this study (patients 5 and 6 were reported previously) (18). All of our patients were male and had a hematocrit >50% and/or a red blood cell count >6 × 10¹²/L on two or more consecutive visits, an arterial oxygen saturation >95% on at least two measurements, and a blood
urea nitrogen >100 mg/dl or serum creatinine level >10 mg/dl. All patients had liver function tests carried out each month during dialysis therapy. None had pulmonary disease and all denied prior recombinant human EPO therapy. α-fetoprotein level, P50 measurement, and liver and renal sonograms were performed in all patients. Carboxyhemoglobin level was determined in the smokers.

**Erythroid Progenitor Cell Assay**

Mononuclear cell fraction of heparinized bone marrow (BM) and peripheral blood (PB) cells were isolated by Ficoll-Hypaque (1.077 g/ml) density gradient centrifugation, and the nonadherent (NA) fraction was separated by adherence technique (19). The NA cells were depleted of E-rosette-forming cells by second Ficoll-Hypaque centrifugation of a mixture of NA cells and sheep red blood cells. T-depleted cells were obtained at the interface and cultured in dishes using Iscove’s technique with modification (20,21). Briefly, 5 x 10⁴ NA T-depleted cells were plated in 0.9% methylcellulose, supplemented with Iscove’s modified Dulbecco’s medium, 5 x 10⁻⁵ M 2-mercaptoethanol, 1% bovine serum albumin, 30% fetal calf serum, with or without addition of 1 U EPO (Connaught Laboratory, Willowdale, Ontario, Canada). Dishes were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. Erythroid colonies were scored by their red color on an inverted microscope on day 7 for CFU-E (colony forming unit-erythroid) and day 14 for BFU-E (burst forming unit-erythroid). Well hemoglobinized colonies visible at 14 d of incubation in the absence of added EPO were counted as EPO-independent erythroid colonies. Thirty normal BM samples, and 20 PB from healthy subjects served as control subjects. Four patients with renal failure and anemia served as an additional control group. Informed consent was obtained from each patient and control subject; investigations were approved by the Human Research Committee at Chang Gung Memorial Hospital.

**Determination of Serum EPO Concentration, Serum IGF-I Level, and Serum Intact Parathyroid Hormone**

Blood samples were taken in the morning at initial evaluation and during the subsequent course. Serum was freshly frozen at −70°C until assay. Serum EPO levels were measured by RIA using a commercially available kit (Diagnostic Systems Laboratories, Webster, TX) with rabbit antihuman EPO purified from urine and calibrated according to the World Health Organization 2nd International Reference preparation. The intra-assay coefficient of variation (n = 10) for EPO levels was 9.8%, and the interassay coefficient of variation (n = 10) was 10.5%. Serum IGF-I levels were also determined by RIA with a commercially available kit (Nichols Institute Diagnostics, San Juan Capistrano, CA), using acid-ethanol extraction method to dissociate IGF-I from IGF-binding proteins. The intra-assay coefficients of variation (n = 12) were 7.2% at 40 μg/L IGF-I and 4.2% at 157 μg/L IGF-I, and the respective interassay coefficients of variation (n = 10) were 10.1 and 6.4%. Serum intact parathyroid hormone (iPTH) was measured using a commercial RIA kit (Nichols Institute Diagnostics), with a normal range of 10 to 65 pg/ml. Duplicate tests were performed for each sample. Sera from healthy adults served as controls. We also included an additional control group of ESRD patients with anemia, who had not yet received recombinant human EPO therapy.

**Effects of Sera from Uremic Patients on Normal Erythropoiesis**

Serum was obtained from each patient immediately before regular hemodialysis. A pool of sera from 10 healthy volunteers served as control serum. Serum was heat-inactivated at 56°C for 30 min and stored at −70°C before testing. In the paired experiments, the effect of 10% sera from both patients and control subjects on normal erythroid progenitor (CFU-E) growth was evaluated using erythroid progenitor assay in the presence of 1 U EPO. The amount of fetal calf serum was reduced to 20% to maintain the total serum concentration of 30%. The experiment was repeated on four different normal target marrows for each serum sample. The effect of serum from uremic patients on normal CFU-E colony growth was expressed as a mean percentage of that with the addition of normal serum. In addition, sera from 11 undialyzed ESRD patients with anemia immediately before the onset of regular hemodialysis were also studied for comparison, and sera from another 13 patients undergoing maintenance hemodialysis for at least 6 mo were assayed before and after one hemodialysis session.

**Anti-IGF-I Neutralizing Experiment**

Polyclonal antihuman IGF-I (Pepro Tech, London, United Kingdom) was used in the neutralizing experiment. Sera were preincubated with 10 μg/ml anti-IGF-I overnight before addition to the cultures for in vitro erythroid progenitor assay as described above. The neutralizing effect was expressed as the percentage of CFU-E number with the addition of anti-IGF-I to that without anti-IGF-I in the presence of uremic serum in normal marrow culture.

**Statistical Analyses**

Because the sample size was small, comparison of variables (BFU-E numbers, serum EPO, and IGF-I levels) between two groups was made by the nonparametric Wilcoxon rank sum test. Comparison of the effects of sera from uremic patients and healthy subjects as well as the effects before and after dialysis in the paired experiments was made by Wilcoxon signed rank test. Spearman’s correlation coefficient was used to assess the linear relationship and its variability between two variables.

**Results**

The patient characteristics and laboratory findings at the time of investigation are shown in Tables 1 and 2. The causes of renal failure included: chronic glomerulonephritis (n = 2), diabetes mellitus (n = 1), diabetes mellitus with hypertension (n = 1), and rapidly progressive glomerulonephritis (n = 1). The remaining three patients initially presented with ESRD with bilateral small and contracted kidneys, for which the causes of renal failure could not be defined. None had splenomegaly. All had arterial oxygen saturation >95% on repeated tests, and none had an elevated carboxyhemoglobin level in smokers. The observed reticulocyte counts ranged from 0.8 to 2.8%, but the absolute reticulocyte counts were increased in the presence of erythrocytosis. Lactate dehydrogenase and haptoglobin levels were all within normal limits. The leukocyte alkaline phosphatase scores were normal in five patients, low in one, and slightly elevated in two. All patients had normal liver function tests and an α-fetoprotein level below 5 ng/ml. Liver sonography revealed a normal liver without tumor or cyst in all patients. Absence of marrow iron stores was found in patients 3 and 5, who also had low serum ferritin levels.

Renal sonography showed that four patients had ACDK, with bilateral multiple renal cysts of various sizes in patients 1, 2, and 3, and multiple cysts in the graft kidney in patient 4. The sizes of the kidneys with cystic lesions were small in patients
1 and 2, and normal in patient 3; patient 4 had a slightly enlarged renal graft. Patients 2 and 3 had undergone renal transplant before, but both had graft removal due to rejection. They had been on maintenance dialysis 2 and 3 yr, respectively, before the development of erythrocytosis. Patient 4 received renal transplantation in December 1987, which was followed by chronic rejection; multiple renal cysts in the graft kidney were found 28 mo later, the renal cysts progressively enlarged, while both native kidneys remained small and contracted without cysts in the subsequent sonography follow-up. The hematocrit levels of patient 4 ranged between 50.3 and 59.7% in the following 8 yr. He has been on regular hemodialysis again since May 1995 when the hematocrit level was 53.1%.

Sonography of the kidneys showed small kidneys without tumors, hydronephrosis, or cysts in patients 5, 6, and 7; the duration of hemodialysis before the discovery of erythrocytosis was 45, 50, and 35 mo, respectively. Patient 8 underwent renal transplant in September 1989. He developed erythrocytosis in July 1990, and his graft function began to deteriorate due to graft rejection in July 1991, which progressed to renal failure in February 1996 when hemodialysis was restarted. His hematocrit levels remained elevated. Renal sonography of patient 8 revealed small and contracted native kidneys with a single small cyst measuring 0.8 cm in the left kidney that had been present before transplantation, and no cyst in the renal graft. Patients 5, 6, 7, and 8 did not have leukocytosis or thrombocytosis. P50 and B12 levels were all within normal limits, and no known causes of secondary erythrocytosis were found in the four patients of non-ACDK during a follow-up time ranging from 14 to 56 mo.

Erythroid Progenitor Culture Assays

Table 3 shows the results of in vitro EPO-dependent BFU-E growth. The number of BFU-E was significantly increased in ESRD patients with erythrocytosis than in normal controls \( (P = 0.021 \text{ for BM; } P = 0.05 \text{ for PB}) \), whereas there was no statistical difference in the BM BFU-E numbers between ESRD patients with erythrocytosis and ESRD with anemia \( (P = 0.5) \) or between ESRD with anemia and normal controls \( (P = 0.092) \). In the absence of exogenous EPO in cultures, none formed EPO-independent erythroid colonies in ESRD patients with erythrocytosis or anemia, or in normal controls.
Table 2. Laboratory findings at the time of evaluation in patients with chronic renal failure and erythrocytosisa

<table>
<thead>
<tr>
<th>Patient</th>
<th>BUN/Cr (mg/dl)</th>
<th>AST/ALT (U/L)</th>
<th>Hb (g/dl)</th>
<th>Hct (%)</th>
<th>RBC (X1012/L)</th>
<th>MCV (fl)</th>
<th>WBC (X109/L)</th>
<th>Platelet (X109/L)</th>
<th>O2 Sat (%)</th>
<th>RBC Mass (ml/kg)</th>
<th>Bone Marrow Iron Stores</th>
<th>Serum Ferritin (ng/ml)</th>
<th>Serum iPTH (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94/17.8</td>
<td>29/24</td>
<td>17.7</td>
<td>54.7</td>
<td>6.23</td>
<td>2.8/174</td>
<td>87.8</td>
<td>9.5</td>
<td>159</td>
<td>96.2</td>
<td>28.6</td>
<td>Normal</td>
<td>566</td>
</tr>
<tr>
<td>2</td>
<td>86/13.9</td>
<td>22/32</td>
<td>18.4</td>
<td>55.5</td>
<td>6.60</td>
<td>1.0/66</td>
<td>85.5</td>
<td>5.5</td>
<td>128</td>
<td>97.4</td>
<td>37.7</td>
<td>Normal</td>
<td>158</td>
</tr>
<tr>
<td>3</td>
<td>79/13.7</td>
<td>19/22</td>
<td>17.4</td>
<td>56.2</td>
<td>7.12</td>
<td>1.4/100</td>
<td>78.9</td>
<td>6.0</td>
<td>190</td>
<td>97.7</td>
<td>48.4</td>
<td>Absent</td>
<td>24.5</td>
</tr>
<tr>
<td>4</td>
<td>82/13.9</td>
<td>10/17</td>
<td>18.2</td>
<td>58.2</td>
<td>7.20</td>
<td>0.8/58</td>
<td>80.9</td>
<td>8.6</td>
<td>147</td>
<td>95.8</td>
<td>39.2</td>
<td>ND</td>
<td>19.5</td>
</tr>
<tr>
<td>5</td>
<td>104/16.0</td>
<td>24/30</td>
<td>16.2</td>
<td>50.6</td>
<td>5.80</td>
<td>1.1/64</td>
<td>87.2</td>
<td>8.1</td>
<td>149</td>
<td>97.0</td>
<td>ND</td>
<td>Absent</td>
<td>37</td>
</tr>
<tr>
<td>6</td>
<td>83/13.5</td>
<td>33/36</td>
<td>17.6</td>
<td>52.8</td>
<td>5.76</td>
<td>1.2/69</td>
<td>91.6</td>
<td>7.6</td>
<td>172</td>
<td>95.0</td>
<td>ND</td>
<td>Mildly decreased</td>
<td>128</td>
</tr>
<tr>
<td>7</td>
<td>72/16.6</td>
<td>9/13</td>
<td>17.3</td>
<td>50.9</td>
<td>5.68</td>
<td>0.9/51</td>
<td>89.6</td>
<td>7.2</td>
<td>259</td>
<td>97.9</td>
<td>30.3</td>
<td>Increased</td>
<td>105</td>
</tr>
<tr>
<td>8</td>
<td>101/8.6</td>
<td>32/31</td>
<td>19.3</td>
<td>59.0</td>
<td>6.10</td>
<td>1.5/92</td>
<td>96.7</td>
<td>8.5</td>
<td>153</td>
<td>95.6</td>
<td>46.5</td>
<td>Increased</td>
<td>4289</td>
</tr>
</tbody>
</table>

a Patients: BM (n = 7), BFU-E (mean ± SD) 536 ± 363; PB (n = 8), BFU-E (mean ± SD) 136 ± 66. Control subjects, ESRD with anemia: BM (n = 4), BFU-E (mean ± SD) 362 ± 133. Normal subjects: BM (n = 30), BFU-E (mean ± SD) 271 ± 160; PB (n = 20), BFU-E (mean ± SD) 88 ± 77. BFU-E, burst forming unit-erythroid; ESRD, end-stage renal disease; BM, bone marrow; PB, peripheral blood; ND, not done.

Table 3. Erythroid progenitor (BFU-E) growth in patients with ESRD and erythrocytosisa

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>BM</td>
<td>PB</td>
</tr>
<tr>
<td>1</td>
<td>318</td>
</tr>
<tr>
<td>2</td>
<td>654</td>
</tr>
<tr>
<td>3</td>
<td>249</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>651</td>
</tr>
<tr>
<td>6</td>
<td>600</td>
</tr>
<tr>
<td>7</td>
<td>1190</td>
</tr>
<tr>
<td>8</td>
<td>88</td>
</tr>
</tbody>
</table>

a Patients: BM (n = 7), BFU-E (mean ± SD) 536 ± 363; PB (n = 8), BFU-E (mean ± SD) 136 ± 66. Control subjects, ESRD with anemia: BM (n = 4), BFU-E (mean ± SD) 362 ± 133. Normal subjects: BM (n = 30), BFU-E (mean ± SD) 271 ± 160; PB (n = 20), BFU-E (mean ± SD) 88 ± 77. BFU-E, burst forming unit-erythroid; ESRD, end-stage renal disease; BM, bone marrow; PB, peripheral blood; ND, not done.

Serum EPO and IGF-I Levels and Effects of Sera from Patients with ESRD on in Vitro Normal Marrow CFU-E Growth

The serum EPO concentrations of patients with ACDK were increased in three patients and constantly normal in one, whereas two of the four patients with non-ACDK had normal or low serum EPO levels and one (patient 6) had a persistently elevated value on repeated assays with comparable hematocrit values during the follow-up course. Patient 5 had fluctuating titers, being normal initially but increasing later in the subsequent measurements. There was no correlation between serum EPO levels and hematocrit values in ESRD patients with erythrocytosis (r = -0.07143, P = 0.87) and uremic anemic patients (r = -0.07762, P = 0.43), respectively.

In the presence of exogenous 1 U EPO, 10% sera from patients with ESRD and erythrocytosis stimulated the normal marrow to give rise to CFU-E colonies 114 to 156% (mean ± SD 135 ± 18%) of those formed by 10% normal sera in predialyzed and regularly dialyzed patients, respectively. The differences in the effects of sera on normal CFU-E growth were statistically significant between ESRD patients with erythrocytosis and predialyzed uremic anemic patients (P = 0.0003) or between ESRD patients with erythrocytosis and chronic dialyzed anemic patients (P = 0.0002). No statistical difference was found between predialyzed sera and sera from those on maintenance dialysis in patients with ESRD and anemia (P = 0.092). Sera assayed in the same individuals with ESRD and anemia before and after one hemodialysis session showed that the effects on CFU-E growth after dialysis were 82 to 136% (mean ± SD 103 ± 16%) of those supported by normal sera in predialyzed and regularly dialyzed patients, respectively. The results of neutralizing experiments showed that the
blocking effects of anti-IGF-I on CFU-E growth in the presence of sera from ESRD patients with erythrocytosis and anemia were not different \((P = 0.8972)\). The stimulatory effects of sera from ESRD with erythrocytosis could partially be nullified by the addition of anti-IGF-I in patients 1, 7, and 8 (Table 4). The neutralizing effect of anti-IGF-I was negatively correlated with serum IGF-I level \(\left( r = -0.6667, P = 0.0710 \right) \), whereas there was no such correlation in ESRD patients with anemia \(\left( r = -0.08571, P = 0.8717 \right) \).

**Discussion**

All eight patients in the present series were male; four patients had ACDK. The diagnosis of ACDK in our patients was established by renal ultrasonography and/or computed tomography, which showed multiple cysts in both native kidneys or renal graft. Although ACDK is a common complication in patients with ESRD on maintenance dialysis (23–25), erythrocytosis has rarely been described in patients with ACDK (10). For patients with ACDK, the cysts usually developed in nontransplanted patients and rarely in the renal graft (26). Three of our four patients with ACDK had prior renal transplants with their native kidneys retained. Multiple cysts developed in the native kidneys in two patients after removal of the rejected grafts, and in the transplanted kidney in another one who also had graft rejection. Erythrocytosis is a well-known complication after renal transplantation, but it usually occurred in patients with excellent allograft function (6,7). Unlike the postrenal transplant erythrocytosis, these three patients had erythrocytosis when they resumed dialysis therapy after redeveloping terminal renal failure following chronic rejection. For the four patients with non-ACDK, all had severe anemia at the initiation of hemodialysis, and they developed erythrocytosis during the course of maintenance dialysis. There were no known extrarenal causes of secondary erythrocytosis, including cardiopulmonary disease and presence of abnormal hemoglobin with high affinity for oxygen. Repeated determinations of arterial blood oxygen content precluded that hypoxia played a role. Other investigators found that hepatitis or hepatocellular carcinoma might be accompanied by an increase in hematocrit and EPO levels in patients with ESRD on dialysis (27–31). Normal liver enzymes and \(\alpha\)-fetoprotein levels as well as normal liver sonograms excluded the possibility that liver disease was the cause of erythrocytosis in our patients.

Our patients with ACDK had elevated or normal EPO levels. These results were comparable to those of serum EPO concentrations in patients with secondary polycythemia of renal cystic disease.

**Table 4. Serum hormone levels and effects of sera from ESRD patients with erythrocytosis on normal bone marrow CFU-E growth**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Serum EPO Level (U/L)</th>
<th>Serum IGF-I Level ((\mu g/L))</th>
<th>% of EPO-Dependent CFU-E with 10% Patient Serum</th>
<th>((%)) Anti-IGF-I Neutralizing Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACDK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7.7 (7.1, 8.2)(^d)</td>
<td>470.3</td>
<td>114</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>65.9 (44.9)</td>
<td>333.0</td>
<td>114</td>
<td>99</td>
</tr>
<tr>
<td>3</td>
<td>55.7 (21.5)</td>
<td>247.5</td>
<td>152</td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>88.4 (41.9, 60.2)</td>
<td>249.8</td>
<td>156</td>
<td>112</td>
</tr>
<tr>
<td>Non-ACDK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>12.5 (47.4, 82.1)</td>
<td>135.0(^e)</td>
<td>145</td>
<td>104(^e)</td>
</tr>
<tr>
<td>6</td>
<td>120.0 (298, 124)</td>
<td>202.5</td>
<td>126</td>
<td>96</td>
</tr>
<tr>
<td>7</td>
<td>9.1 (4.5, 4.3)</td>
<td>416.3</td>
<td>153</td>
<td>72</td>
</tr>
<tr>
<td>8</td>
<td>12.6 (8.5)</td>
<td>362.3</td>
<td>118</td>
<td>66</td>
</tr>
<tr>
<td>Patients ((n = 8))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>46.5 ± 42.8</td>
<td>302.1 ± 113.1</td>
<td>135 ± 18%</td>
<td>88 ± 17%</td>
</tr>
<tr>
<td>range</td>
<td>7.7 to 120</td>
<td>135.0 to 470.3</td>
<td>114 to 156%</td>
<td>66 to 112%</td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>normal subjects ((n = 40))</td>
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</tr>
<tr>
<td>mean ± SD</td>
<td>9.28 ± 2.41</td>
<td>199.2 ± 42.7</td>
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<td></td>
</tr>
<tr>
<td>range</td>
<td>5.80 to 15.30</td>
<td>97.9 to 384.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESRD with anemia ((n = 105))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>24.2 ± 42.4</td>
<td>174.7 ± 84.0</td>
<td>84 ± 13%</td>
<td>90 ± 16%</td>
</tr>
<tr>
<td>range</td>
<td>4.1 to 240</td>
<td>73.1 to 337.5</td>
<td>26 to 106%</td>
<td>67.0 to 112.0%</td>
</tr>
</tbody>
</table>

\(^a\) CFU-E, colony forming unit-erythroid; EPO, erythropoietin; IGF-I, insulin-like growth factor I; ACDK, acquired cystic disease of the kidney.

\(^b\) Expressed as a percentage of those with 10% normal serum.

\(^c\) Expressed as the percentage of CFU-E number with the addition of anti-IGF-I of that without anti-IGF-I in the presence of 10% uremic serum.

\(^d\) Numbers in parentheses indicate levels of subsequent measurements during follow-up.

\(^e\) Measured with an EPO level of 82.1 U/L.
disease or renal cell carcinoma but without renal failure (32,33). Edmunds et al. found that the development of ACDK was associated with increased EPO levels in patients receiving regular hemodialysis, but no patient in their study had a hemoglobin greater than 15 g/dl (34). Shalhoub et al. described two patients with erythrocytosis and increased serum EPO levels accompanying secondary cyst formation in long-term hemodialysis (10). The stimulation of EPO production in ACDK may be attributed to local hypoxia of remnant kidney tissue caused by the pressure of cystic formation, which bears a resemblance to the cysts in autosomal dominant polycystic kidney disease (9,35). Increased EPO production under conditions of erythrocytosis in our patients also supports the possibility of disturbed oxygen sensor function in the diseased kidneys of chronic renal failure as suggested by Eckardt et al. (35). On the other hand, patients with ACDK and erythrocytosis were not necessarily associated with an increased serum EPO level as seen in patient 1. The results of previous studies on the correlation between hematocrit level and degree of cystic disease or kidney size were conflicting (25,36). We and others found that the kidney sizes of ACDK might be reduced, normal, or enlarged (38). We also found that serum EPO levels did not correlate with the degree of renal cystic disease. The serum EPO levels of non-ACDK patients were consistently normal in two, markedly increased in one, and fluctuated in another one on repeated measurements. The cause of increased EPO secretion in patients 5 and 6 was not identified in the long-term follow-up.

The present study confirmed that serum EPO levels vary widely in uremic anemic patients and EPO levels have no correlation with the hematocrit levels (39,40). We also found that there was no correlation between serum EPO levels and hematocrit values in ESRD patients with erythrocytosis. None of our ESRD patients had EPO-independent BFU-E colonies that are characteristic of polycythemia vera (41,42). In the presence of EPO in cultures, the numbers of BFU-E colonies in patients with ESRD and erythrocytosis were significantly increased compared with those of normal subjects. These findings suggest that their erythroid progenitors exhibited an increased sensitivity to EPO. However, ESRD patients with erythrocytosis or anemia did not show a statistical difference in the BFU-E number, which made the increased sensitivity of BFU-E to EPO an important factor responsible for erythrocytosis unlikely.

It has been demonstrated that IGF-I enhanced human erythroid progenitor growth in vitro and IGF-I could substitute for EPO as a stimulator of erythropoiesis (43–46). Brox et al. have shown that EPO-like factor from an anephric patient's serum with a hematocrit of 38 to 40% and a low-to-normal EPO level was able to stimulate late erythropoiesis (47). This erythroid cell-stimulating factor was later purified and characterized as IGF-I by Congote et al. (48). Three of our patients did not have elevated EPO levels on serial assays; we thus measured the serum IGF-I levels in ESRD patients with erythrocytosis and anemia. Serum IGF-I levels in adult uremic anemic patients determined by RIA have been found to be low (49,50). Our results showed that serum IGF-I levels in ESRD and anemia were lower than normal but did not reach statistical signifi-


cance. In contrast, patients with ESRD and erythrocytosis had significantly higher serum IGF-I levels than those of healthy subjects, or patients with ESRD and anemia. These findings suggest that IGF-I might play a role in the regulation of erythrocytosis in our patients with ESRD and erythrocytosis, especially in those who did not have elevated serum EPO values. Urena et al. also found that IGF-I stimulated erythropoiesis in uremic patients with severe hyperparathyroidism (51). They demonstrated that serum IGF-I concentrations were well correlated with hematocrit levels; however, unlike our patients, their patients had a low hematocrit level with a mean of 28.1 ± 1.7%. To prove that the elevation in IGF-I was causally related to the erythrocytosis in our patients, we performed a neutralizing experiment by adding the anti-IGF-I to the in vitro erythroid progenitor assay in the presence of patients’ sera. Our results showed that anti-IGF-I could partially abolish the erythropoietic action of serum on CFU-E growth. The correlation coefficient between serum IGF-I level and the blocking effect of anti-IGF-I in this small series was not so high, which was partly attributed to the addition of exogenous EPO in the cultures and the presence of other possible inhibitors or stimulators in the uremic serum.

Erythropoietic inhibitors in sera from ESRD patients with anemia have been demonstrated by others (52–56). Our results also showed that sera from undialyzed patients with ESRD and anemia inhibited CFU-E formation in normal human marrow cultures. Some investigators found that uremic sera contained dialyzable inhibitors of in vitro erythropoiesis (52,54,56). The effects of sera from uremic anemic patients on normal marrow CFU-E growth were comparable before and after hemodialysis in the present study. In contrast, we found that sera from patients with ESRD and erythrocytosis produced a significant stimulation of normal marrow CFU-E growth. These findings showed that their sera did not have an inhibiting effect, but rather enhanced erythropoietic activity. Since the effect of sera from ESRD with erythrocytosis was the net effect of stimulation and inhibition, the increased erythropoietic effect in the sera of ESRD patients with an elevated EPO or IGF-I levels might have overcome the uremic inhibitors.

In conclusion, we reported the clinical and laboratory results of eight patients with a rare combination of disease: ESRD and erythrocytosis. The present results suggest that IGF-I could be an important factor regulating erythropoiesis in patients with ESRD and erythrocytosis, especially in those who did not have an increased EPO production.

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