We have studied serum immunoreactive erythropoietin (SIE) levels in 28 patients with sickle cell anemia (SCA) without renal insufficiency and in 17 patients with nonhemoglobinopathy anemias of comparable severity using a sensitive radioimmunoassay procedure. An exponential relationship between SIE level and degree of anemia was noted in all patients. However, in nonhemoglobinopathy anemia, a sharp rise in the SIE level occurred as hemoglobin (Hb) levels fell below about 12 g/dL, whereas in sickle cell patients the increase was not marked until hemoglobin fell to about 9 g/dL. The response was more blunted in older SCA patients than in younger ones. A linear regression model relating SIE level to Hb level, presence/absence of SCA, and age explained 63% of the variation in SIE. We conclude that the serum erythropoietin levels in SCA increased at a lower hemoglobin concentration and are of a lower magnitude than that of the other anemias.

© 1986 by Grune & Stratton, Inc.

From the Division of Hematology, Department of Medicine, Albert Einstein College of Medicine, Bronx, NY; the Departments of Biochemistry and Pediatrics, University of Chicago; and the Department of General Medicine, Department of Medicine, Columbia University School of Medicine, New York.

Submitted Nov 19, 1984; accepted June 25, 1985.

Supported by Grants No. HL-21016 and HL-21676 from the National Institutes of Health, US Public Health Service.

Address reprint requests to Dr Judith B. Sherwood, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, NY 10461.

© 1986 by Grune & Stratton, Inc.

0006-4971/86/0701-0007$03.00/0

Sickle Cell Anemia Patients Have Low Erythropoietin Levels for Their Degree of Anemia

By Judith B. Sherwood, Eugene Goldwasser, Robert Chilcote, L. David Carmichael, and Ronald L. Nagel

A CONSIDERABLE NUMBER of clinical and experimental observations demonstrate the inverse relationship between the erythropoietin level in plasma and urine and the level of tissue oxygenation. A direct correlation between elevated erythropoietin levels and the degree of hypoxic stimulus has been shown as well as suppression of erythropoietin levels with hyperoxia or red cell plethora. The inverse relationship between the red cell mass and hemoglobin concentration, and the circulating erythropoietin titer, has been amply demonstrated.

The erythropoietin response in several anemias has been more difficult to establish, in part because of the lack of precision of the bioassay methods heretofore available and because of parameters of the specific anemia such as Hb-O₂ affinity, renal damage, etc.

We have studied the correlation between erythropoietin levels and hemoglobin concentrations in patients with sickle cell anemia who have red cells with low oxygen affinity as well as in patients with anemias not associated with hemoglobinopathies. We have used the sensitive and specific radioimmunoassay for erythropoietin, which allows the accurate measurement of normal and depressed levels of human serum erythropoietin without prior concentration of samples. Finally, we have examined the effect of age on the Hb level–erythropoietin relationship.

MATERIALS AND METHODS

Subjects. Serum was obtained from patients with anemias of various causes. 28 patients had sickle cell disease (ten adults and 18 children and young adults). Two additional subjects with Sβ⁺-thal were also studied. Of the 18 children and young adults with sickle cell disease, 12 were males and six females, and they had a mean age of 9.2 ± 5.5 years with a range of 1 to 17. Their mean hemoglobin level was 7.6 ± 1.6 g/dL with a range of 3.1 to 10.1 g/dL; the reticulocyte count was 19.4 ± 10.1% with a range of 0.1% to 36.3%. All patients had a BUN less than 20 mg/dL. Of the ten adult patients with sickle cell anemia, seven were females and three males; they had a mean age of 31.0 ± 6.6 years with a range of 22 to 40 years. Thirteen patients had anemias not associated with hemolysis nor with any hemoglobinopathy; three patients had chronic lymphocytic leukemia, one had chronic myelocytic leukemia, four had multiple myeloma, and five had anemias not associated with hemolytic malignancy. All of the patients with non-sickle cell anemia had normal BUN levels (mean, 16.1 ± 3.42 mg/dL) and serum creatinine levels less than 2 mg/dL. Four patients had hemolytic anemias not associated with hemoglobinopathy. None of these patients had been transfused and all had a BUN less than 20 mg/dL. The two pediatric patients with Sβ⁺-thal had a mean age of 7 ± 2 years, a mean hemoglobin level of 7.6 ± 0.65 g/dL, a reticulocyte count of 15.2% ± 4.8%, a mean corpuscular volume (MCV) of 71.5 ± 0.5 µm³, a mean corpuscular hemoglobin (MCH) of 24.8 ± 0.45 pg, a mean corpuscular hemoglobin concentration (MCHC) of 35% ± 0.95%, and a bilirubin level of 0.7 ± 0.7 mg/dL. All hematologic and other laboratory data of these patients are shown in Table 1.

Blood was allowed to clot on ice for two hours. The serum was separated by centrifugation and stored at −20 °C until assay.

Assay procedures. Serum immunoreactive erythropoietin (SIE) was measured in each serum sample by using the radioimmunoassay procedure described by Sherwood and Goldwasser. Erythropoietin was labeled with 125I by using the water-insoluble oxidizing agent lodogen (Pierce Chemical Co, Rockville Ill). The iodinated tracer was incubated with erythropoietin standard or 100 µL of serum sample and with antiserum in a phosphate buffer diluent. The anti-erythropoietin antibody was used at a dilution that gave a bound-to-total antibody-erythropoietin ratio (B/T) between 0.20 to 0.30. The antibody-erythropoietin complex was separated from the free hormone either by precipitation with ammonium sulfate by using a modification of the method developed by Stanley or by use of the second antibody technique previously described.

Statistical analysis. Preliminary examination of the data revealed a nonlinear relationship of the SIE level to varying degrees of anemia, with nonuniform variances proportional to the mean SIE level (Table 1). Therefore, a log-linear model with a nonzero asymptote was used because of the lack of evidence for saturation of the antibody-erythropoietin complex. The model of the form

\[ y = b_0 + b_1 \text{Hb} + e \]

was chosen, and the other patient variables (age, presence of sickle cell disease, and other hematologic characteristics) were evaluated for their contribution by standard regression analysis. The asymptote c was set at 0 µU/mL, approximating the normal mean ± 2 SD as established in this laboratory’s assay. This procedure allowed us to select variables that significantly affected the SIE level, with approximately normal distribution of error terms and an F value for the model of 22.0 (P < 2 × 10⁻⁴).
ERThropoietin in Sickle Cell Anemia

Table 1. Laboratory Values of Anemic Patients

<table>
<thead>
<tr>
<th>Anemia</th>
<th>No. of Patients</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>Retic (%)</th>
<th>HbF (%)</th>
<th>MCV (μm³)</th>
<th>MCH (pg)</th>
<th>MCHC (%)</th>
<th>Bilirubin (mg/dL)</th>
<th>EPO (mU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle Cell Anemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.2 ± 5.5 yr (1-17)</td>
<td>18</td>
<td>7.61 ± 1.58</td>
<td>21.14 ± 4.71</td>
<td>19.25 ± 10.1</td>
<td>6.48 ± 6.8</td>
<td>90.17 ± 10.5</td>
<td>32.95 ± 5.5</td>
<td>36.25 ± 2.8</td>
<td>2.97 ± 2.8</td>
<td>192.52 ± 291.1</td>
</tr>
<tr>
<td>31 ± 6.6 yr (22-40)</td>
<td>10</td>
<td>8.08 ± 1.46</td>
<td>14.1 ± 2.9</td>
<td>10.3 ± 4.4</td>
<td>5.2 ± 3.2</td>
<td>95.8 ± 9.6</td>
<td>32.9 ± 2.5</td>
<td>34.3 ± 2.2</td>
<td>3.2 ± 1.6</td>
<td>58 ± 63</td>
</tr>
<tr>
<td>Nonhemoglobinopathy</td>
<td>13</td>
<td>9.9 ± 2</td>
<td>28.5 ± 19.1</td>
<td>—</td>
<td>—</td>
<td>93.6 ± 19.1</td>
<td>32.3 ± 6.5</td>
<td>34.6 ± 1.9</td>
<td>2 ± 3.5</td>
<td>420 ± 366</td>
</tr>
<tr>
<td>Hemolytic, Nonhemoglobinopathy</td>
<td>4</td>
<td>8.88 ± 3.92</td>
<td>26.40 ± 11.4</td>
<td>32.17 ± 30.3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>395.0 ± 159.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Abbreviations: Hct, hematocrit; EPO, erythropoietin; Hb, hemoglobin. Values are expressed as mean ± SD. Numbers in parentheses are the range of values. Hematologic indexes (MCV, MCH, MCHC) were obtained by the Coulter Counter (Coulter Electronics, Hialeah, Fla). Erythropoietin values were determined in the radioimmunoassay.

RESULTS

SIE levels were measured in 17 patients with anemias of various causes not associated with sickle cell anemia, other hemoglobinopathies, or renal disease (including four patients with hemolytic anemia) and in 28 patients with sickle cell anemia (ten adults and 18 children); of these, the 17 non-sickle cell anemia patients and 26 sickle cell anemia patients for whom all variables were recorded were used in the analysis. The two patients with Sβ-thal were analyzed separately. We found that Hb level, age, and sickle cell anemia status predicted 63% (R²) of the variance in SIE (Figs 1 and 2), with $F_{3,39} = 22.0$ ($P < 2 	imes 10^{-4}$). The significance of the contribution of the individual terms was tested, and presence/absence of sickle cell anemia was highly significant ($P < .0004$); the effect of age, however, was significant only among sickle cell anemia patients ($P = .011$). The adult sickle cell anemia patient with 220 mU/mL of SIE appeared to be an outlier; however, there were no major alterations in the model when this patient was excluded, and there appeared to be no technical reasons to question the accuracy of the SIE determination.

The mean SIE level of the anemic patients was significantly higher than the normal value of $21 ± 6$ (X ± SD) mU/mL previously found with this radioimmunoassay. The 17 patients with nonhemoglobinopathy-associated anemias had the highest levels: $414 ± 327$ mU/mL of serum (range, 21 to 1,300 mU/mL), with a mean hemoglobin concentration of $9.7 ± 2.5$ g/dL (range, 6.1 to 12.8 g/dL). The mean SIE level of the four patients with hemolytic anemias not associated with hemoglobinopathy was $395 ± 195$ mU/mL (range, 220 to 610 mU/mL), a value that did not differ significantly from the levels found in the other nonhemoglobinopathy anemias (Fig 1). The mean value found for the patients with sickle cell anemia was $144 ± 234$ mU/mL of serum (range 12 to 1,300 mU/mL), with a mean hemoglobin concentration of $7.8 ± 1.5$ g/dL (range, 3 to 11 g/dL). As shown in Fig 2, the sickle cell anemia patients have less SIE at any level of hemoglobin; SIE levels rise only when considerably lower Hb levels are reached, as compared with non-sickle cell anemia patients.

The adult sickle cell anemia patients had a mean SIE level of $56 ± 63$ mU/mL of serum (range, 12 to 220 mU/mL)
with a mean hemoglobin concentration of 8.1 ± 1.5 g/dL (range, 6 to 11 g/dL). The children with sickle cell anemia, with a similar hemoglobin concentration of 7.6 ± 1.6 g/dL (range, 3 to 10 g/dL), had a mean SIE level of 193 ± 292 mU/mL of serum (range, 27 to 1,300 mU/mL). When a child with 3 g/dL hemoglobin and an erythropoietin level of 1,300 mU/mL was excluded, the mean SIE level for the children was 127 ± 96 mU/mL, and the mean hemoglobin level was 7.9 ± 1.1 g/dL. Analysis of covariance comparing the adult and pediatric sickle cell patients showed that the average erythropoietin level was significantly different at P < .02, even when the child with the very low hemoglobin level (3.1 g/dL) and high erythropoietin level (1,300 mU/mL) was not included in the computation.

The two children with Sβ-thal had a mean Hb level of 7.6 ± 0.65 g/dL and an SIE level of 84.2 ± 33.8 mU/mL (50.5, 118). When they were included in the regression model, there was no significant alteration in its parameters, and its strength actually increased slightly (R² = 65%).

DISCUSSION

SIE levels were determined in pediatric and adult patients with sickle cell disease and in patients with nonhemoglobinopathy anemias of various causes, including hemolytic anemias by using our sensitive radioimmunoassay procedure.7 An exponential relationship between the SIE level and the hemoglobin concentration was noted in all patients. In patients with nonhemoglobinopathy anemias, we observed a drastically increased slope at a hemoglobin concentration of approximately 11 g/dL or less (Figs 1 and 2). This hemoglobin level can be considered as a threshold. The data presented here on the inverse relationship between hemoglobin concentration and immunoreactive erythropoietin levels in anemias other than sickle cell (Fig 1) are in general agreement with the previously published data based on biologic activity.12

The relationship between Hb levels and SIE in the sickle cell anemia patients departs from this since these patients exhibit elevated levels of erythropoietin only at a hemoglobin concentration below about 9 g/dL (Fig 2). The patients with nonhemoglobinopathy-associated anemias and other types of hemolytic anemias do not exhibit this hemoglobin threshold and have levels of SIE that are greater than that of the sickle cell patients (Fig 2). Our observation that patients with sickle cell anemia produce less immunoreactive erythropoietin at a given hemoglobin concentration than do patients with nonhemoglobinopathy anemias (hemolytic and nonhemolytic) agrees with a previous observation using the biologic assay.13

We conclude that the relationship between SIE and Hb levels in sickle cell anemia can be described by an exponential curve that is significantly displaced to the left. In addition, the level of SIE reached by these patients is also significantly lower than that observed in other anemias. Analysis of the variables involved in the relationship between SIE and Hb levels by using a linear regression model indicated that age, Hb level, and presence or absence of anemia explained 63% (R²) of the variance in the SIE level. Of special interest is the effect of age, as pediatric sickle cell anemia patients tended to have significantly higher SIE levels than adults. The potential mechanism for the low SIE levels in sickle cell disease include interference with the renal synthesis of erythropoietin and the displacement to the right of the O₂ equilibrium curve.

The well-known right shift in the oxygen equilibrium curve would facilitate oxygen delivery to the tissues, decreasing the hypoxic burden of a given level of anemia. Sickle cells have a higher p50 because of the intrinsically lower affinity for oxygen of the hemoglobin S polymer14 and because of the increase in intracellular 2,3-diphosphoglycerate acid levels.15 In addition, kinetic factors (decreased uptake and diffusion of O₂) contribute to this anomaly in vivo.16

Renal tissue damage in these patients might compromise the synthesis of erythropoietin or the hypoxia sensor presumed to be located in this organ. The renal damage, which differs from renal insufficiency, involves a concentration defect, progressive vascular damage particularly to the medulla, and structural abnormalities of the glomeruli and tubules.17 In favor of this interpretation is the age dependency of the level of SIE in sickle cell anemia patients (Fig 2). Individuals with chronic renal failure have SIE levels that are higher than normal, but lower than expected for the degree of anemia.18 In addition, Morgan et al13 observed that in adults with sickle cell anemia the concentration of erythropoietin was correlated positively with creatinine clearance and inversely with the BUN and that hemoglobin concentrations were depressed in the presence of minor degrees of renal insufficiency. Further work is necessary to establish definitively the mechanism of the decreased erythropoietin response in the patient with sickle cell anemia.

ACKNOWLEDGMENT

We are grateful for the help of Dr Christine Lawrence and Dr Parviz Lalazari in securing samples for this work. The technical contributions of Sandra Ewert and Annette Gardner and the secretarial assistance of Elizabeth Ezzone are gratefully acknowledged.

REFERENCES

Sickle cell anemia patients have low erythropoietin levels for their degree of anemia

JB Sherwood, E Goldwasser, R Chilcote, LD Carmichael and RL Nagel