Serum Level of Erythropoietin in Anemias Associated with Chronic Infection, Malignancy, and Primary Hematopoietic Disease

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ABSTRACT The serum level of erythropoietin was measured in 31 patients with anemia secondary to chronic infection or malignancy and compared with erythropoietin levels in 23 patients with iron-deficiency anemia and 14 patients with primary hematopoietic diseases. Erythropoietin levels varied directly with the degree of anemia in patients with iron deficiency or primary hematopoietic disorders. There was no correlation of erythropoietin and the degree of anemia in patients with chronic infection or malignancy and the erythropoietin levels were significantly lower than in patients with iron deficiency or primary hematopoietic disease and the same degree of anemia. A major factor in the anemia of chronic disorders is a decrease in levels of erythropoietin.

INTRODUCTION

The anemia of chronic disorders as described by Cartwright (1) is characterized by decreased plasma iron and iron-binding capacity, despite increased iron stores in the reticuloendothelial cells of the marrow. There is an impairment of release of iron into the plasma from the reticuloendothelial stores (2), a modest decrease in red cell survival (3), and failure to increase red cell production sufficiently (4). Recently, we (5) reported a reduction of serum levels of erythropoietin in patients with anemia associated with rheumatoid arthritis and suggested that the anemia of other chronic disorders may reflect an inappropriate erythropoietin response for the degree of anemia.

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The purpose of this paper is to evaluate erythropoietin levels in chronic infection, malignancy, and primary hematopoietic disease, e.g., leukemia and aplastic anemia. Erythropoietin-blocking factors were evaluated by an in vivo neutralization test with the use of the erythropoietin bioassay.

METHODS

Patients. Three groups of patients were studied: group I, chronic inflammation and malignancy; group II, primary hematopoietic disease; and group III, iron deficiency. All patients were seen at Colorado General Hospital, Denver General Hospital, or the Denver Veterans Administration Hospital, between 1968 and 1970. The final discharge diagnosis and laboratory parameters of the 31 patients with anemia of chronic disorders, group I, are listed in Table I. Six of the eight patients with chronic urinary tract infection had indwelling catheters. All patients with coexisting renal insufficiency (serum creatinine $>$ 1.4 mg/100 ml or creatinine clearance $<$ 60 ml/min) were excluded from the study. All patients with carcinoma had evidence of metastasis, but bone marrow biopsy did not show malignant cells in any patient. 30 of the 31 patients had adequate bone marrow sections for evaluation of iron stores. Increased iron was found in 19 patients, normal stores in 7, and absent iron in 4 patients.

A variety of primary myeloid hematopoietic diseases was represented in group II: 7 patients with aplastic or hypoplastic anemia of unknown etiology, 3 patients with refractory sideroblastic anemia, 2 patients with acute granulocytic leukemia, a case of agnogenic myeloid metaplasia, and a 52 yr old male with homozygous sickle cell anemia. All 14 patients in this group had increased iron in the bone marrow.

Group III patients had iron-deficiency anemia diagnosed on the basis of a hypochromic, microcytic blood smear; low 15% iron saturation; and, in all patients so evaluated, absent iron stores on Prussian blue stains of bone marrow. Iron deficiency was due to gastrointestinal bleeding in 22 patients, and red cell fragmentation from cardiac valve dysfunction in one patient. Patients with significant liver disease were excluded from this group. This group was reported in a previous communication (5).

Erythropoietin assay. Serum samples were collected from the majority of patients in the fasting state during the morn-

TABLE I

Diagnosis and Laboratory Parameters of 31 Patients with Anemia of Chronic Disorders (Group I)

Patient and diagnosis	Hct.	Serum iron	Total iron-binding capacity	Marrow iron*	Serum erythropoietin
	%	μg/100 ml	μg/100 ml		% ^{59}Fe in RBC
Infection					
1. J. M., urinary infection	23	12	210	A	0.31
2. L. C., urinary infection	29	105	240	N	0.50
3. L. W., urinary infection	30	65	195	I	0.30
4. F. Mc., urinary infection	28	32	195	I	0.90
5. C. O., urinary infection	27	16	159	I	1.24‡
6. J. P., urinary infection	30	54	234	I	2.42‡
6. S. J., urinary infection	22	32	240	A	0.78
8. I. Z., urinary infection	31	18	260	I	0.75
9. S. K., tuberculosis	24	44	102	I	0.42
10. J. N., tuberculosis	33	29	187	A	1.98‡
11. D. Mc., pneumonia	25	11	198	I	0.98
12. F. B., pneumonia	33	15	246	N	0.66
13. E. T., septic arthritis	31	60	210	I	0.17
14. C. F., staphylococcal abscess	29	36	165	I	1.38‡
Lymphoma					
1. H. S., Hodgkin's 4B	30	32	195	N	0.74
2. W. R., Hodgkin's 4B	29	39	185	N	0.72
3. A. C., Hodgkin's 4B	19	42	179	I	2.62‡
4. D. H., Hodgkin's 4B	32	48	205	I	1.18‡
5. L. A., Hodgkin's 4B	32	37	200	I	1.41‡
6. W. M., lymphosarcoma 4B	33	56	215	I	1.89‡
7. A. K., lymphosarcoma 4B	34	47	204	I	0.79
8. R. C., lymphosarcoma 3B	34	40	180	A	0.85
Carcinoma					
1. A. Z., lung	33	45	165	I	0.45
2. A. B., lung	23	33	190	N	0.43
3. H. B., renal	20	12	70	I	3.13‡
4. N. C., gastric	32	25	210	—	0.73
Miscellaneous					
1. J. V., recurring pulmonary embolism	31	27	265	N	1.59‡
2. R. L., chronic phlebitis, alcoholism	28	43	264	N	2.27‡
3. V. Z., Buerger's disease	33	49	288	I	0.45
4. S. T., gout, nursing home	24	89	267	I	0.42
5. F. S., chronic alcoholism	21	46	221	I	0.44
Normal	42–49	60–180	250–450	N	

* Posterior iliac crest, biopsy section, stained with Prussian blue; A = absent, N = normal (1+ or 2+); I = increased (3+ or 4+).

‡ Significant increase when compared to control, as determined by Student's *t* test.

ing hours from 8 to 11 a.m. All samples of serum were frozen for a period of 1–4 wk before assay. Serum was collected instead of plasma in order to use the serum sample in bone marrow culture experiments. We have found an insignificant different in erythropoietin levels measured in serum or plasma. The method for erythropoietin assay was basically the method of Cotes and Bangham (6). Virgin female CFI [1] mice weighing approximately 23 g were placed in a hypobaric chamber, and maintained at a simulated altitude of 18,000 ft continually for 3 wk. 5 days after removal from the hypobaric chamber, 0.5 ml of serum was injected subcutaneously into each mouse. On the 7th day, $^{59}FeCl_3$ (0.5 μCi) in 0.5 ml of saline was injected intraperitoneally. The animals were killed on the 10th day, heparinized blood (0.5 ml) from each animal placed in a plastic counting vial with an equal volume of heparinized saline, and the radioactivity determined. The ^{59}Fe uptake was calculated as a percentage of the total dose, with the use of a

[1] Carworth Farms, Portage, Mich.

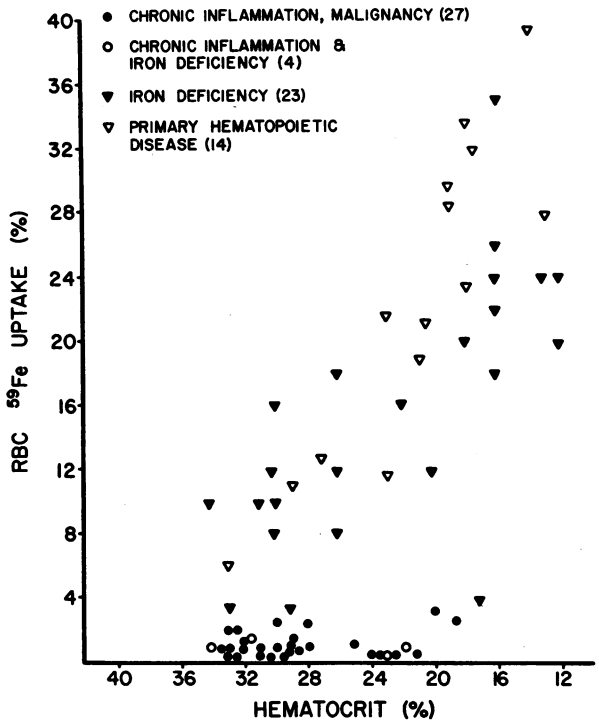

FIGURE 1 Correlation of serum level of erythropoietin with the venous hematocrit.

formula for blood volume as 7% of the body weight. A microhematocrit was determined on each animal, and animals with a hematocrit less than 60% were discarded from the calculations. Groups of four or five animals were used for each assay, and controls of normal saline and 0.125 U of a commercial erythropoietin preparation[2] (ESF) were assayed simultaneously. The erythropoietin preparation had been standardized against erythropoietin standard B by the commercial laboratory,[2] and demonstrated a log-dose response in our assay. Mean values of ^{59}Fe incorporation into red cells in test groups of mice were compared to the control group injected with saline and the significance of any differences determined by Student's *t* test.

In vivo neutralization test. The patient's serum was incubated overnight at 37°C with an equal volume of erythropoietin standard suspended in saline. Cultures of this mixture after the 12 hr incubation period were negative for bacterial growth. A volume of 0.5 ml, containing 0.125 U of erythropoietin, was injected into the test group and compared to a control of 0.125 U of erythropoietin without the patient's serum.

RESULTS

11 of the 31 patients in group I with anemia associated with chronic inflammation and malignancy had significantly elevated erythropoietin levels when compared with the saline controls (Fig. 1). However, there was no correlation between the level of erythropoietin and the severity of anemia in the 31 patients (slope − 0.0223, correlation coefficient − 0.1311). In sharp contrast, the erythropoietin activity in unconcentrated serum was directly related to the severity of anemia in the 14 patients in group II with primary hematopoietic disease (slope − 1.5641, correlation coefficient − 0.8802) and the 23 patients in group III with iron-deficiency anemia (slope − 0.7224, correlation coefficient 0.6836) (Fig. 1). There was no significant difference by covariant analysis between the slopes of groups II and III and, therefore, both groups were combined (slope − 1.0200, correlation coefficient 0.7293).

The erythropoietin levels in group I were significantly lower ($P < 0.001$) than in groups II and III. Four patients in group I had coexisting iron deficiency (Fig. 1); levels of erythropoietin in these patients were similar to the level in other patients with chronic disorders and unlike the markedly elevated levels in pure iron-deficiency anemia.

Sera from two patients with anemia associated with chronic disorders were evaluated for erythropoietin-blocking factors by an in vivo neutralization test (Table II). Blocking factors were not demonstrated in either serum sample.

DISCUSSION

The serum level of erythropoietin was directly related to the severity of anemia in patients with primary hematopoietic disorders and in patients with iron-deficiency anemia. Similar findings were reported by Movassaghi, Shore, and Hammond (7, 8) in patients with iron deficiency and in patients with congenital hypoplastic anemias, thalassemia major, and sickle cell anemia. In these studies, as well as in our study, correlation of the erythropoietin level with the hematocrit was less evident at mild levels of anemia (hematocrit greater than 30%); probably a reflection of the bioassay insensitivity. The elegant study by Adamson (9) clearly showed a direct relation-

TABLE II

In Vivo Neutralization Test for Erythropoietin Blocking Factor(s)

Test	RBC ^{59}Fe uptake
	%
Saline	0.66 ±0.02*
ESF (0.125 U)‡	7.12 ±0.11
Patient C. O. and ESF (0.125 U)‡	0.99 ±0.02
	6.10 ±0.21
Patient M. C. and ESF (0.125 U)‡	0.67 ±0.01
	4.79 ±0.68

* SEM.

‡ Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada.

[2] Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada.

ship of the severity of anemia with the log of erythropoietin excretion in normal patients made anemic by bleeding. In his study, urinary concentrates were assayed in polycythemic, protein-depleted mice, a more sensitive method for determining erythropoietin production than the serum assay employed in our study.

Erythropoietin levels were elevated in 11 of 31 patients with anemia secondary to chronic infection or malignancy. The degree of elevation was significantly less than in patients with iron-deficiency anemia or primary hematopoietic disorders with the same degree of anemia. The failure of the serum erythropoietin level to increase in proportion to the degree of anemia in patients with chronic infection or malignancy is similar to our findings in patients with anemia secondary to rheumatoid arthritis (5). Four patients with coexisting iron deficiency had significantly lower levels of erythropoietin than if their anemia had been due solely to iron deficiency. This finding is consistent with the clinical observation that iron-deficient patients with anemia of chronic infection or malignancy respond poorly to iron therapy.

The low level of erythropoietin in anemias of chronic disorders may represent an artifact caused by blocking factors that inhibit the erythropoietin bioassay. We were unable to demonstrate a blocking factor in the sera of two patients by an in vivo neutralization test. Although this finding makes an anti-erythropoietin factor unlikely, we cannot exclude the possibility of a tightly bound complex of erythropoietin–erythropoietin antibody without excess antibody in the serum. In addition, a serum factor that blocks the effect of erythropoietin at a cellular level, similar to the nuclear antibody described by Krantz and Kao (10), should have been detected by the neutralization test unless the antibody is directed specifically against human cells and does not react with mouse erythroblasts.

The recent concepts of intra-erythrocytic adaptation to anemia have not been evaluated in chronic disorders. An exaggerated 2,3-diphosphoglycerate (DPG) response to anemia (hypoxemia) in these patients would cause an increase in oxygen release from hemoglobin and, thereby, a reduction in the hypoxic stimulus for erythropoietin production at the tissue level. Although unlikely, this possibility needs to be evaluated in these patients.

The inadequate level of erythropoietin in these patients and in patients with rheumatoid arthritis (5) suggests a common abnormality in all patients with the anemia of chronic disorders and could reflect either a rapid turnover or impaired synthesis of the hormone. Evidence to evaluate these possibilities is lacking. The ability of cobalt (11), erythropoietin (12), and hypoxemia (12) to correct the anemia of infection is consistent with either explanation.

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