Bone Marrow Erythropoiesis in the Anemia of Infection, Inflammation, and Malignancy

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Abstract A major factor in the anemia of infection, inflammation, and malignancy is a relative failure of the bone marrow to increase erythropoiesis in response to a shortened red cell survival. The possible causes for this diminished marrow response are: (a) a reduced production of erythropoietin, or, (b) impaired bone marrow response to erythropoietin. In this report studies were performed on 6 normals, 13 patients with anemia from infection or inflammation, and 18 patients with anemia caused by advanced malignancy. Serum erythropoietin activity was measured using the post-hypoxic, polycythemic mouse assay. Assessment of bone marrow response to erythropoietin was made by measuring 59Fe-heme synthesis in bone marrow suspensions cultured for 3 days with and without the addition of erythropoietin. The results showed that marrow heme synthesis was increased in erythropoietin-treated cultures as compared with saline control cultures by 66±8% (mean ±SE) in normals, 101±10% in patients with infection or inflammation, and 31±5% in malignancy. Serum erythropoietin levels were consistently diminished relative to expected levels for the degree of anemia in the infection-inflammatory group, but not in malignancy. In these patients, plasma inhibitors to the biological activity of erythropoietin were not detected in vitro. These studies suggest that another factor to consider in the anemia of malignancy is a decreased bone marrow response to erythropoietin. In the anemia of infection-inflammation, marrow response to erythropoietin is normal, but serum levels of erythropoietin are decreased relative to the degree of anemia.

Introduction

Anemia commonly occurs in chronic infection, inflammation, and malignancy (1–4). This anemia is characterized by: (a) decreased plasma iron and iron-binding capacity; (b) impaired release of reticuloendothelial iron into the plasma; (c) modest decrease of red cell survival; and (d) relative failure of bone marrow to increase red cell production (1–7). These clinical similarities have resulted in the classification of these anemias in a single category, the anemia of chronic disorders (ACD)3 (8).

The purpose of this study was to determine whether the relative erythroid hypoproliferation in ACD was due to either inadequate production of erythropoietin, the physiological hormone regulating erythropoiesis, or to diminished bone marrow responsiveness to secreted hormone.

Methods

Subjects. Male patients with ACD were selected for study according to the following criteria: (a) a normocytic or slightly microcytic anemia (hemoglobin less than 13 g/100 ml), associated with decreased serum iron and iron-binding capacity; (b) infection, rheumatoid arthritis, or malignancy of at least 3 wk duration; and (c) no other cause for the anemia discovered after clinical and laboratory investigation. Patients with a blood urea nitrogen above 25 mg/100 ml, total serum bilirubin above 1.6 mg/100 ml, or evidence of marrow aplasia were excluded from the study. The vast majority of these patients had normal or low reticulocyte counts, normal or increased marrow iron stores, elevated sedimentation rates, and hypoalbuminemia.

Patients were divided into four groups according to clinical and laboratory criteria: Group I consisted of six normal volunteers (mean age of 52 yr). Group II consisted of 13 patients with anemia secondary to active infection or inflammation (mean age of 55 yr). No neoplasms were noted in this group. Group III consisted of 18 patients with advanced malignancy (mean age of 65 yr). Three patients had received localized (lung or lumbar spine) radiotherapy 3–6 mo before study. 15 of 18 patients were alive 1 mo after the study. Even though pa-

1 Abbreviation used in this paper: ACD, anemia of chronic disorders.
tients with malignancy had evidence of metastasis, malign-
nant cells were not found on examination of bone mar-
row aspirates or biopsies. Group IIIA, a supplemental
sample group for the serum erythropoietin study, consisted of
an additional 13 adult male patients with anemia and malign-
nancy (lung cancer \( n = 2 \), prostate cancer \( n = 2 \), gas-
trointestinal malignancy \( n = 2 \), multiple myeloma \( n = 3 \),
and chronic leukemia \( n = 41 \) ). Group IV, an anemic
control group for the serum erythropoietin study, consisted of
adult males with anemia due to nonmalignant, non-
infectious hematopoietic disease (iron deficiency \( n = 11 \)
and folate deficiency \( n = 33 \) ).
In a supplemental study, bone marrow cultures were also
done on six male patients with malignancy (renal \( n = 1 \),
lung \( n = 1 \), Hodgkin's disease \( n = 1 \), lymphosar-
coma \( n = 2 \), and multiple myeloma \( n = 15 \) ), but withou
anemia (hemoglobin above 13.5 g/100 ml).

Procedures. Hemoglobin, hematocrit, reticulocyte counts,
and bone marrow staining of siderocytes were per-
computed by previously described methods (9). Bone marrow smears
were prepared and stained with Wright-Giemsa, and quant-
tative nucleated cell counts were performed by differential
counting techniques (9). Bone marrow cultures were mea-
sured by the method of Rutstein, Ingento, and Reynolds
(11). Serum iron and iron-binding capacity were measured
by the method of Goodwin, Murphy, and Guillemette (12).
Bone marrow cultures were prepared as previously de-
scribed (10, 13). In brief, the procedure entailed aspirating
approximately 3 ml of bone marrow from the posterior iliac
crest or sternum into syringes containing 50 U/ml heparin.
The patients who had received prior radiotherapy,
the bone marrow aspirate was obtained from a site out-
side of the field of radiotherapy. The marrow cells
were dispersed in sterile Hank's balanced salt solution
(Grand Island Biological Co., Grand Island, N. Y.) by passage
through a 10-ml pipette 10 times (Falcon Plastics, Los
Angeles, Calif.) and then centrifuged at 1,000 g for 10
min at 4°C. The plasma and marrow fat which floated
to the top of the liquid were removed. The cells were
washed a second time in Hanks balanced salt solution, the
supernate was discarded, and the cells were suspended in a
final medium consisting of 20% plasma from a normal
donor of AB blood type, 20% sterile precolostrum calf
serum (Colorado Serum Co., Denver, Colo.), 60% NCTC-
109 (Microbiological Associates, Inc., Bethesda, Md.), with
the addition of 20 U/ml of penicillin G and 20 \( \mu \)g/ml of streptomycin.
The nucleated cell concentration was ad-
justed to between 5,000-9,000/mm\(^3\) and 0.8 ml of the final
cell suspension was added to 35 \( \times \) 10-mm tissue culture
dishes (Falcon Plastics). Sheep plasma erythropoietin con-
aining 4.79 U/mg protein (lot 3002, Connaught Medical
Research Laboratory, University of Toronto, Ontario,
Canada) was suspended in NCTC-109 and aliquots, diluted
to 1 and 3 U/ml, were stored at \(-40°C\). The biological
activity of erythropoietin was confirmed by repeated testing
in an in vitro rat-marrow assay system (14). 0.2 ml of the
erythropoietin stock solutions was added to culture dishes
to provide final concentrations of 0.2 and 0.6 U/ml,
respectively. Preliminary experiments had indicated that
these concentrations of sheep erythropoietin were optimal for
stimulating in vitro \(^{57}Fe\) incorporation into heme. 0.2
ml of NCTC-109 was added to control cultures. All cul-
ture dishes were performed in triplicate. Stationary marrow
suspensions were cultured at 37°C in an atmosphere of 5%
CO\(_2\) and 95% air with high humidity (CO\(_2\) Incubator,
model 3221, National Appliance Co., Portland, Ore.). At
68 h of incubation, \(^{57}Fe\) as sterile ferrous citrate (Mal-
linkrodt Chemical Works, St. Louis, Mo.) with specific activity
of 4.8 mCi/mg, which had been preincubated with fetal
calf serum as a source of unbound transferrin, was added
to the culture dishes in a final volume of 0.1 ml
(final concentration of 2 \( \mu \)Ci/ml). The cultures were re-
icubated for 4 h, transferred to polycarbonate tubes, and
washed twice with cold phosphate-buffered saline. The
supernate was discarded and the packed cells were lysed by
the addition of 3.0 ml of Drabkin's solution diluted to
one-third of its usual concentration (13). Heme was ex-
tracted from the lysate by the method of Teale (15) as
modified by Krantz, Moore, and Zaentz (13) using cyclo-
hexanone. \(^{57}Fe\) in the upper phase was counted in an
automated gamma well-type scintillation counter (Nuclear-
Chicago Corp., Des Plaines, Ill.). The total number of
counts obtained with each specimen was such that
the counting error was less than 3% (95% confidence limits).
The mean "intra-assay" coefficient of variation of tripli-
cates was 12%. The maximum stimulation of heme
synthesis by erythropoietin in cultures containing 0.2 or 0.6
U of erythropoietin/ml was compared with the control
cultures and expressed as percentages above control. Re-
results were analyzed for significance of differences using
the unpaired Student's \( t \) test and the analysis of variance
(16).

Serum erythropoietin assay. Sterile serum was collected
at the time of bone marrow aspiration and stored at
\(-40°C\). Erythropoietin was assayed by a modification of
the posthypoxic, polycythemic mouse assay method of
Lange, Simmons, and McDonald (17). In this assay, virgin
CF-1 female mice were exposed to reduced oxygen tension
for 4 wk in cages fabricated with dimethyl silicone rubber
membranes (General Electric Co., Chemical & Medical Div.,
Schenectady, N. Y.). 4 days after removal from hyper-
poxia the mice received intraperitoneal injections of 0.5 ml
of sterile human serum. 2 days later 0.5 \( \mu \)Ci of \(^{57}Fe\) as
ferrous citrate diluted in saline was injected into the tail
vein of mice. The incorporation of \(^{57}Fe\) into mouse periph-
eral red blood cells was measured 48 h later by obtaining
heparinized blood via cardiac puncture and measuring radio-
activity in an aliquot of lysed whole blood (17). Groups of
four or five animals were used for each assay. Results
from mice with hematocrits of less than 55% were excluded
from calculations. Laboratory controls, consisting of saline
and 0.2 U erythropoietin (Connaught Medical Research
Laboratory), were tested with each assay group of mice.
The slopes relating the serum erythropoietin content to
the hemoglobin level were calculated by least square, best
fit approximations (16).

In vitro assay for plasma inhibitors to erythropoietin.
Heparinized patient plasma (0.1 ml) was incubated with
either 0.1 ml of NCTC-109 or 0.1 ml of erythropoietin
in NCTC-109 (containing 0.1 U of erythropoietin) for
2 h at 37°C. Each incubation mixture was then added to
0.8 ml of rat bone marrow cell suspension containing 1.3 \( \times \)
10\(^6\) nucleated cells. The rat marrow cell suspensions were
prepared from the femurs of male Wistar rats as de-
scribed by Hrinda and Goldwasser (18). These rat mar-
row cells were cultured in triplicate for 20 h in an atmos-
phere of 5% CO\(_2\) and 95% air with high humidity.
Ferrous citrate (\(^{57}Fe\)) was then added to each culture and,
after an additional 4 h of incubation, the cultures were
terminated and heme extracted as described above. The
incorporation of iron into heme was calculated as previously
described (10).

Erythropoiesis in Anemia of Chronic Disorders
1135
TABLE I

Laboratory Data in Anemia of Chronic Disorders

<table>
<thead>
<tr>
<th></th>
<th>Hb</th>
<th>Serum Fe</th>
<th>TIBC*</th>
<th>Albumin</th>
<th>Marrow-nucleated red cells</th>
<th>Marrow normoblast B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I, normals</td>
<td>16.2±0.7</td>
<td>102±10</td>
<td>340±23</td>
<td>4.6±0.3</td>
<td>31±7</td>
<td>14±3</td>
</tr>
<tr>
<td>Group II, infection-inflammation</td>
<td>10.9±1.9</td>
<td>53±17</td>
<td>285±56</td>
<td>3.3±0.9</td>
<td>22±7</td>
<td>8±5</td>
</tr>
<tr>
<td>Group III, malignancy</td>
<td>10.3±2.1</td>
<td>53±32</td>
<td>234±38</td>
<td>2.9±0.6</td>
<td>26±14</td>
<td>11±7</td>
</tr>
</tbody>
</table>

Mean±SD.
* TIBC, total iron-binding capacity.

RESULTS
As shown in Table I, mean hemoglobin, serum iron, total iron-binding capacity, and serum albumin concentrations were below normal in group II (infection-inflammation) and group III (malignancy). The percentages of marrow-nucleated erythroid cells and cells of normoblast B type (polychromatophilic) were also below normal in groups II and III.

In vitro bone marrow response to erythropoietin. Dose-response curves from typical experiments involving one patient each in groups II and III are depicted in Fig. 1. The maximum stimulation of heme synthesis by erythropoietin in marrow culture is listed in Table II. Maximum stimulation of 55Fe incorporation into heme (heme synthesis) was observed in most patients in groups I, II, and III at an erythropoietin concentration of 0.2 U/ml. The mean stimulation (±SE) of heme synthesis was 66.0±7.7% in group I (normal), 101.3±10.4% in group II, and 30.9±4.7% in group III (Fig. 2). Using the analysis of variance, the differences among the groups were significant at P < 0.001 level (16). The differences between groups I and III, and between groups II and III were significant at P < 0.001 (Student’s t test). The difference between groups I and II was significant at P < 0.05.

A second bone marrow aspiration and culture was done on two patients in group III (patients 30 and 35) and repeated poor responses to erythropoietin in vitro were noted. The in vitro bone marrow response to erythropoietin was also tested in six anemic patients with malignancy. The erythropoietin-induced stimulation of heme synthesis in this group ranged between 32–142%, with a mean of 78.5±17.9% (SE). The difference in erythropoietin effect between the malignant group with anemia (group III) and the malignant group without anemia is significant at P < 0.005.

![Figure 1](http://www.jci.org)  Typical effect of erythropoietin (0.2 and 0.6 U/ml) on 55Fe incorporation into heme in marrow cultures from a patient with inflammation (patient 7) and a patient with malignancy (patient 35). Results include the mean ±SE of triplicate cultures.

![Figure 2](http://www.jci.org)  Effect of erythropoietin (0.2 or 0.6 U/ml) on 55Fe incorporation into heme in marrow cultures from patients in groups I, II, and III. Stimulation of heme synthesis is recorded as percentages above saline controls. Results include the mean±SE of each group.

1134  S. Zucker, S. Friedman, and R. M. Lysik
**TABLE II**

*Clinical Data, Marrow Response to Erythropoietin, and Serum Erythropoietin Levels in Anemia of Chronic Disorders*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Duration of disease</th>
<th>Hb</th>
<th>% increase above control</th>
<th>% <strong>Fe</strong> incorp. ± SE in mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group I</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-6</td>
<td>31-63</td>
<td>Normal controls</td>
<td>0</td>
<td>15.2-17.0</td>
<td>46*-93*</td>
<td>0.23-0.41</td>
</tr>
<tr>
<td><strong>Group II</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>Rheumatoid arthritis</td>
<td>12</td>
<td>12.2</td>
<td>95*</td>
<td>1.39±0.20</td>
</tr>
<tr>
<td>8</td>
<td>56</td>
<td>Rheumatoid arthritis</td>
<td>55</td>
<td>12.5</td>
<td>129*</td>
<td>0.48±0.07</td>
</tr>
<tr>
<td>9</td>
<td>50</td>
<td>Rheumatoid arthritis</td>
<td>24</td>
<td>12.0</td>
<td>133*</td>
<td>0.71±0.10</td>
</tr>
<tr>
<td>10</td>
<td>79</td>
<td>Pneumonia and pyelonephritis</td>
<td>4</td>
<td>7.0</td>
<td>62*</td>
<td>0.65±0.27</td>
</tr>
<tr>
<td>11</td>
<td>76</td>
<td>Perirectal abscess</td>
<td>1</td>
<td>12.4</td>
<td>179* (0.6E)</td>
<td>—</td>
</tr>
<tr>
<td>12</td>
<td>75</td>
<td>Pneumonia</td>
<td>1</td>
<td>10.5</td>
<td>151* (0.6E)</td>
<td>1.91±0.29</td>
</tr>
<tr>
<td>13</td>
<td>55</td>
<td>Bacterial endocarditis</td>
<td>1</td>
<td>8.3</td>
<td>117*</td>
<td>0.40±0.06</td>
</tr>
<tr>
<td>14</td>
<td>53</td>
<td>Bacteremia</td>
<td>1</td>
<td>8.6</td>
<td>91*</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td>15</td>
<td>49</td>
<td>Bacteremia</td>
<td>1</td>
<td>10.3</td>
<td>72*</td>
<td>0.31±0.08</td>
</tr>
<tr>
<td>16</td>
<td>43</td>
<td>Osteomyelitis</td>
<td>4</td>
<td>11.8</td>
<td>68* (0.6E)</td>
<td>0.27±0.04</td>
</tr>
<tr>
<td>17</td>
<td>40</td>
<td>Cellulitis</td>
<td>2</td>
<td>11.2</td>
<td>78*</td>
<td>0.32±0.05</td>
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<tr>
<td>18</td>
<td>33</td>
<td>Toxoplasmosis</td>
<td>1</td>
<td>12.7</td>
<td>61* (0.6E)</td>
<td>—</td>
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<tr>
<td>19</td>
<td>23</td>
<td>Viremia</td>
<td>1</td>
<td>12.3</td>
<td>81*</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td><strong>Group III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>79</td>
<td>Rectal adenocarcinoma</td>
<td>12</td>
<td>7.1</td>
<td>58*</td>
<td>11.37±1.82</td>
</tr>
<tr>
<td>21</td>
<td>79</td>
<td>Rectal adenocarcinoma</td>
<td>8†</td>
<td>9.6</td>
<td>22*</td>
<td>0.81±0.15</td>
</tr>
<tr>
<td>22</td>
<td>81</td>
<td>Colon adenocarcinoma</td>
<td>2</td>
<td>6.0</td>
<td>43*</td>
<td>11.80±3.20</td>
</tr>
<tr>
<td>23</td>
<td>79</td>
<td>Colon adenocarcinoma</td>
<td>24</td>
<td>11.4</td>
<td>48*</td>
<td>—</td>
</tr>
<tr>
<td>24</td>
<td>74</td>
<td>Colon adenocarcinoma</td>
<td>5</td>
<td>10.5</td>
<td>49* (0.6E)</td>
<td>3.29±0.53</td>
</tr>
<tr>
<td>25</td>
<td>54</td>
<td>Colon adenocarcinoma</td>
<td>6</td>
<td>11.0</td>
<td>55* (0.6E)</td>
<td>0.88±0.14</td>
</tr>
<tr>
<td>26</td>
<td>53</td>
<td>Duodenal adenocarcinoma</td>
<td>3</td>
<td>10.0</td>
<td>22*</td>
<td>1.34±0.14</td>
</tr>
<tr>
<td>27</td>
<td>54</td>
<td>Gastric adenocarcinoma</td>
<td>3</td>
<td>10.5</td>
<td>49*</td>
<td>4.15±0.75</td>
</tr>
<tr>
<td>28</td>
<td>59</td>
<td>Bronchogenic carcinoma</td>
<td>12†</td>
<td>11.0</td>
<td>34* (0.6E)</td>
<td>0.62±0.06</td>
</tr>
<tr>
<td>29</td>
<td>54</td>
<td>Bronchogenic carcinoma</td>
<td>12†</td>
<td>9.7</td>
<td>16 (0.6E)</td>
<td>—</td>
</tr>
<tr>
<td>30</td>
<td>58</td>
<td>Lung adenocarcinoma</td>
<td>2</td>
<td>12.5</td>
<td>8* (0.6E)</td>
<td>1.32±0.21</td>
</tr>
<tr>
<td>31</td>
<td>59</td>
<td>Mesothelioma</td>
<td>6</td>
<td>11.7</td>
<td>49*</td>
<td>2.58±0.41</td>
</tr>
<tr>
<td>32</td>
<td>81</td>
<td>Prostate carcinoma</td>
<td>12</td>
<td>11.8</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>33</td>
<td>56</td>
<td>Anaplastic carcinoma</td>
<td>1</td>
<td>12.0</td>
<td>39*</td>
<td>—</td>
</tr>
<tr>
<td>34</td>
<td>50</td>
<td>Metastatic carcinoma to liver</td>
<td>4</td>
<td>12.7</td>
<td>4</td>
<td>—</td>
</tr>
<tr>
<td>35</td>
<td>78</td>
<td>Reticulum cell sarcoma</td>
<td>3</td>
<td>8.4</td>
<td>12 (0.6E)</td>
<td>0.62±0.10</td>
</tr>
<tr>
<td>36</td>
<td>78</td>
<td>Lymphosarcoma</td>
<td>&gt;12</td>
<td>6.5</td>
<td>45*</td>
<td>0.39±0.05</td>
</tr>
<tr>
<td>37</td>
<td>41</td>
<td>Hodgkin’s disease</td>
<td>3</td>
<td>12.6</td>
<td>6</td>
<td>—</td>
</tr>
</tbody>
</table>

* Significant difference between erythropoietin and control cultures, $P < 0.05$.
† Previous radiotherapy.
§ Unless otherwise stated (0.6E unit), maximum stimulation of heme synthesis was noted at an erythropoietin concentration of 0.2 U/ml.

**Serum erythropoietin assay.** In the erythropoietin assay, the saline control and 0.2 U of erythropoietin resulted in a **Fe incorporation into red cells of mice of 0.2-0.5 and 6.0-7.2%, respectively.**

Serum erythropoietin levels were above normal levels (> 0.5% **Fe uptake in mice) in 4 of 11 patients in group II, 22 of 25 patients in group III-IIIA, and 13 of 14 patients in group IV. Serum erythropoietin levels were compared with the hemoglobin measurements in groups III–IIIA and IV (Fig. 3). In group IV (iron deficiency and folate deficiency anemia) the serum erythropoietin activity correlated inversely with the hemoglobin level (slope = -1.204, r = -0.740, $P < 0.005$). Similarly, in group III–IIIA (malignancy),

**Erythropoiesis in Anemia of Chronic Disorders**
the serum erythropoietin activity correlated inversely with the hemoglobin level (slope = -1.287, \( r = -0.689, P < 0.001 \)). There was no significant difference (t = 0.347, \( P > 0.70 \)) between the slope of group III–IIIA and group IV (16). In group II there was no correlation between the serum erythropoietin activity and the hemoglobin level (slope = +0.095, \( r = +0.027, P > 0.7 \)).

**Figure 3** Correlation of serum level of erythropoietin with the venous hemoglobin concentration. The solid line indicates the slope for patients with iron deficiency and folate deficiency. The dashed line denotes the slope for all patients with malignancy (least square, best fit approximations).

**Assay for plasma inhibitors to erythropoietin.** The effectiveness of the in vitro neutralization test for detecting inhibitors to erythropoietin was demonstrated by adding rabbit antiserum to erythropoietin to a known concentration of purified sheep erythropoietin (Connaught Medical Research Laboratory). The antiserum (sufficient to neutralize 0.2 U of erythropoietin in the in vivo mouse assay) (19) completely neutralized the biological activity of 0.1 U of erythropoietin (Table III).

The plasmas from one normal person, two patients with the anemia of infection, and two patients with the anemia of malignancy were evaluated for the presence of inhibitors to erythropoietin. In the 24-h rat marrow culture described above, erythropoietin in NCTC-109 resulted in an increase of heme synthesis of 92% above the control. The stimulatory effect of erythropoietin persisted in the presence of plasmas from a normal person and patients with the anemias of infection and malignancy (Table III). No plasma inhibitors to erythropoietin were noted. Plasma from anemic patients, in the absence of exogenous erythropoietin also enhanced heme synthesis.

**Table III**

<table>
<thead>
<tr>
<th>Addition</th>
<th>Patient plasma or NCTC-109</th>
<th>Incorporation of Fe into heme</th>
<th>% increase above control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythropoietin, 0.1 U</td>
<td>ng/1.3 ( \times 10^6 ) marrow cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>% increase above control</td>
<td></td>
</tr>
<tr>
<td>NCTC-109</td>
<td>-</td>
<td>3.9 \pm 0.2 (SE)</td>
<td>92.3</td>
</tr>
<tr>
<td>NCTC-109</td>
<td>+</td>
<td>7.5 \pm 0.5</td>
<td></td>
</tr>
<tr>
<td>NCTC-109 + antiserum to erythropoietin</td>
<td>-</td>
<td>4.3 \pm 0.2</td>
<td></td>
</tr>
<tr>
<td>NCTC-109 + antiserum to erythropoietin</td>
<td>+</td>
<td>3.5 \pm 0.3</td>
<td>0 (19% inhibition)</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>-</td>
<td>3.0 \pm 0.1</td>
<td></td>
</tr>
<tr>
<td>Normal plasma</td>
<td>+</td>
<td>5.4 \pm 0.3</td>
<td>80.0</td>
</tr>
<tr>
<td>Patient 14</td>
<td>-</td>
<td>4.9 \pm 0.3</td>
<td></td>
</tr>
<tr>
<td>Patient 14</td>
<td>+</td>
<td>9.3 \pm 0.2</td>
<td>89.7</td>
</tr>
<tr>
<td>Patient 16</td>
<td>-</td>
<td>5.8 \pm 0.2</td>
<td></td>
</tr>
<tr>
<td>Patient 16</td>
<td>+</td>
<td>9.0 \pm 0.4</td>
<td>55.1</td>
</tr>
<tr>
<td>Patient 20</td>
<td>-</td>
<td>5.3 \pm 0.2</td>
<td></td>
</tr>
<tr>
<td>Patient 20</td>
<td>+</td>
<td>10.6 \pm 0.5</td>
<td>100.0</td>
</tr>
<tr>
<td>Patient 36</td>
<td>-</td>
<td>7.1 \pm 0.3</td>
<td></td>
</tr>
<tr>
<td>Patient 36</td>
<td>+</td>
<td>14.7 \pm 0.7</td>
<td>92.9</td>
</tr>
</tbody>
</table>

**DISCUSSION**

A major factor in ACD is a relative failure of the bone marrow to increase erythropoiesis in response to a modestly shortened red cell survival (2, 7, 8). Normal bone marrow can rapidly increase the red cell production rate by a factor of five to eight times the basal rate, provided that erythropoietin production increases proportionately and the normal bone marrow responsiveness to the hormone persists (20, 21). In our study of short-term, normal bone marrow cell cultures, exogenous erythropoietin produced a 46-94% increase in heme synthesis, as compared with saline control cultures. In marrow cell cultures from patients with anemia associated with advanced malignancy (without malignant infiltration of bone marrow), erythropoietin stimulated heme synthesis by a mean value of only 31±5% (SE). In contrast, in patients with anemia associated with infection or inflammation, heme synthesis was enhanced by a mean of 101±10% (SE). These two groups of patients were otherwise similar in respect to mean levels of hemoglobin, blood reticulocytes, serum iron, and marrow erythroid cellularity. In another group of patients with malignancy but without anemia, the mean bone marrow response to erythropoietin (79±18%) did not differ significantly from normal (\( P > 0.5 \)). These patients with malign-

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**Note:** Antisera to erythropoietin kindly supplied by Dr. R. D. Lange, University of Tennessee Memorial Research Center and Hospital, Knoxville, Tenn.
nancy without anemia, however, had less advanced disease and fewer surgical procedures than those with malignancy and anemia. Multiple factors, therefore, must be considered in interpreting this data. The degree of marrow hyporesponsiveness to erythropoietin in the anemia of malignancy takes on even greater proportions when contrasted with the hyperresponsive state of marrow from patients with infection or inflammation. Enhanced in vitro marrow response to erythropoietin in inflammation has also recently been demonstrated in our laboratory in anemic rats with turpentine abscesses.* These data suggest that marrow hyporesponsiveness to erythropoietin may be an important factor in the pathogenesis of the anemia of malignancy, but not in the anemia of infection or inflammation. Additional studies, however, will be required to prove this hypothesis. The mechanism for this tumor effect remains conjectural. The production of catabolic products of tumor, the secretion of physiologic inhibitors of erythropoiesis, or some form of metabolic competition have been previously postulated (13, 22, 23). A severe degree of bone marrow unresponsiveness to erythropoietin has been described in polycythemia vera, but not in chronic myelogenous leukemia (10, 24). In polycythemia vera, Krantz postulated the existence of an abnormal erythroid cell line that was functionally autonomous and hence unresponsive to hormonal control (24). Further research will be needed to clarify the relationship between marrow hyporesponsiveness to erythropoietin in cancer and polycythemia vera.

The effects of chronic disease on erythropoietin production in man remains disputed. In most other forms of anemia, as the hemoglobin is depressed below 9–10 g/100 ml, the level of plasma and urine erythropoietin are elevated in a roughly linear fashion (25). Ward, Kurnick, and Pisarczyk have noted significantly lower serum erythropoietin levels in anemia of infection and inflammation as compared to patients with iron deficiency or primary hematopoietic disease (26). Lower than expected levels of erythropoietin in the anemia of infection and inflammation were also noted in the present study. Likewise, in rats with experimental arthritis and mild anemia, Lukens has reported a relative failure in production of biologically active erythropoietin (27). Data on erythropoietin production in malignancy remains controversial. Ward et al. and Firat and Banzon have noted no correlation between the erythropoietin levels and the degree of anemia in malignancy (26, 28). Alexanian, on the other hand, has recently reported that urinary erythropoietin levels in human malignancy were appropriately elevated for the degree of anemia (29). In the study reported here, in most patients with the anemia of malignancy, serum erythropoietin levels were significantly above normal, and furthermore, a linear correlation existed between the degree of anemia and the level of erythropoietin. The increase in serum erythropoietin relative to the degree of anemia was not significantly different in malignancy as compared to a group of anemic patients with iron or folate deficiency. The discrepancy between the results of the current report and those of Ward et al. and Firat and Banzon might be due to differences in patient population. Most of the patients in the latter reports had lymphomas in contrast to the predominance of solid tumors in the current report. Caution should be used, however, in interpreting erythropoietin data from patients with mild forms of anemia (hemoglobin above 10 g/100 ml (25). The nutritional status must also be considered in evaluating erythropoiesis in malignancy since protein starvation, a frequent complication of advanced malignancy, may itself result in depression of erythropoietin production (30–32). The possibility of an inhibitor to erythropoietin in infection or malignancy was also considered in the current study. Using an in vitro neutralization test, blocking factors to erythropoietin were not demonstrated in patients with the anemia of infection or malignancy.

The current study suggests that the mechanism for anemia of infection or inflammation may differ from the anemia of malignancy. In the anemia of infection or inflammation, decreased erythropoietin production would appear to be of central importance. In many patients with the anemia of malignancy, however, erythropoietin production appears to be normal. Another factor that should be considered in the anemia of malignancy is bone marrow hyporesponsiveness to erythropoietin.

The importance of the blockade of reticuloendothelial iron release in the pathogenesis of ACD also remains to be elucidated. Experimental studies, however, have shown that the anemia of inflammation or infection can be corrected by the administration of erythropoietin or testosterone without a concomitant increase in serum iron concentration (27, 33, 34). The supply of erythropoietin or response to erythropoietin, therefore, appears to be the rate-limiting factor in determining red cell production.

**ACKNOWLEDGMENTS**

The authors would like to express their appreciation to Dr. S. Wassertheil-Smoller for statistical advice; to Dr. R. Singer, Ms. H. Bien, Ms. D. Fontanella, and Mr. C. C. Smith for technical assistance; and to Doctors W. H. Crosby, H. W. Fritts, Jr., and L. E. Meiselas for suggestions in preparation of this manuscript.

**Erythropoiesis in Anemia of Chronic Disorders**
REFERENCES


S. Zucker, S. Friedman, and R. M. Lysk