Granulopoietic and Erythropoietic Activity in Patients With Anemias of Iron Deficiency and Chronic Disease

By Tariq Mahmood, William A. Robinson, John E. Kurnick, and Rita Vautrin

The serum levels of granulocyte colony-stimulating factor (CSF) and erythropoietin (Ep) were measured in 16 patients with iron-deficiency anemia and 15 patients with the anemia of chronic disease. Levels of both CSF and Ep in the serum of patients with iron-deficiency anemia had an inverse linear relationship to the level of the packed cell volume (PCV). There was no correlation between PCV and the levels of CSF or Ep in the serum of patients with the anemia of chronic disease. The similarity in the behavior of CSF and Ep in iron-deficiency anemia suggests that they may be influenced by similar control mechanisms or have a common cellular or molecular source.

Recent years have seen rapid advancement in the understanding of the factors regulating hematopoietic cellular proliferation and maturation. Erythropoietin (Ep) is generally accepted as the hormone regulating red cell production. Its level in body fluids is traditionally determined by bioassay in an animal whose endogenous Ep has been suppressed. Less is known about the factors regulating granulocyte production, but the best contender for a true granulopoietic substance is the colony-stimulating factor (CSF). The latter has been so named because of its ability to stimulate granulocyte-macrophage colony formation in semisolid tissue culture systems. The number and size of colonies developing has been shown to be a function of the concentration of CSF present in the system, allowing assay of levels of this material in serum and urine. A body of data has been accumulated to suggest that CSF is a physiologic regulator of granulopoiesis.

Although several materials have been described with colony-stimulating activity (CSA), the most important factor in humans appears to be a sialic acid-containing glycoprotein with a molecular weight of about 45,000 daltons. These biochemical properties of CSF are very similar to those described for Ep. In addition, both migrate electrophoretically between alpha1 globulin and albumin. Despite these biochemical similarities they have different biologic properties. Ep does not stimulate mouse bone marrow to form granulocyte-macrophage colonies, and CSF has no erythropoietic activity. Furthermore, they are separable by concanavalin A-Sepharose column chromatography.

While there is some controversy about the exact organ and cellular source...
of Ep, it is clear that the kidney is the major regulatory organ.\textsuperscript{11} Whether the kidney actually produces the Ep molecule, or if a precursor molecule produced in the liver is transformed into Ep by the kidney has not been fully determined.\textsuperscript{12,14} Recently, data have been provided that the reticuloendothelial system (RES) is a source of extrarenal Ep, and that the Kupffer cells of the liver represent a major source of Ep in the anephric rat.\textsuperscript{15} The major cellular source of CSF in humans appears to be the monocyte-macrophage complex.\textsuperscript{16,18} It has also been noted that CSF can be derived in large amounts from isolated livers and spleens.\textsuperscript{19} These data suggest that both CSF and Ep are derived, at least partially, from the RES. The factor controlling the production of erythropoietin appears largely to be tissue hypoxia.\textsuperscript{20} The control mechanisms for CSF production have not been clearly established. It has been suggested by several authors that regulation involves antigenic stimulation derived from bacterial flora or their products, particularly endotoxin.\textsuperscript{21,22}

Cycling of CSF levels in serum and urine has been noted in patients with chronic cyclic neutropenia.\textsuperscript{23,24} It is of considerable interest that cycling of Ep coinciding with the fluctuations of CSF levels has also been noted in this disorder.\textsuperscript{25} The reasons behind this observation are unknown. These findings, along with biochemical similarities between these two substances, have prompted us to determine the levels of CSF and Ep in patients with iron-deficiency anemia and the anemia of chronic disease to determine if correlations in the levels of these two activities can be found. Anemias of iron deficiency and chronic disease have been chosen because the granulocyte count in both disorders is usually normal. In iron-deficiency anemia the Ep levels are high, and in the anemia of chronic disease they are frequently low.\textsuperscript{26,27} In patients with

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>PCV (%)</th>
<th>MCV (fL)</th>
<th>Serum Fe/TIBC (µmole/liter)</th>
<th>BM Fe* (x 10^9/liter)</th>
<th>CSF† Erythropoietin expressed as percent (^{59})Fe incorporation (normal &lt;1%).</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M Peptic ulcer</td>
<td>31</td>
<td>74</td>
<td>7.32/63.98</td>
<td>Absent</td>
<td>4900</td>
<td>62</td>
</tr>
<tr>
<td>2/F Menorrhagia</td>
<td>24.5</td>
<td>61</td>
<td>10.72/71.49</td>
<td>Absent</td>
<td>8800</td>
<td>62</td>
</tr>
<tr>
<td>3/M Hemorrhoids</td>
<td>32</td>
<td>73</td>
<td>2.14/105.9</td>
<td>Absent</td>
<td>7500</td>
<td>49</td>
</tr>
<tr>
<td>4/M GI bleed (ulcer)</td>
<td>18</td>
<td>80</td>
<td>2.30/82.03</td>
<td>Absent</td>
<td>8200</td>
<td>70</td>
</tr>
<tr>
<td>5/M Osler-Weber-Rendu syndrome</td>
<td>30</td>
<td>68</td>
<td>2.68/80.78</td>
<td>Not done</td>
<td>4600</td>
<td>50</td>
</tr>
<tr>
<td>6/M Colonic polyp</td>
<td>31</td>
<td>77</td>
<td>8.57/100.80</td>
<td>Absent</td>
<td>4200</td>
<td>53</td>
</tr>
<tr>
<td>7/M Peptic ulcer</td>
<td>18</td>
<td>51</td>
<td>0.89/78.46</td>
<td>Not done</td>
<td>7800</td>
<td>64</td>
</tr>
<tr>
<td>8/M Peptic ulcer</td>
<td>28</td>
<td>60</td>
<td>1.78/67.56</td>
<td>Absent</td>
<td>6300</td>
<td>37</td>
</tr>
<tr>
<td>9/F Fibroid, uterus</td>
<td>24</td>
<td>65</td>
<td>2.14/68.09</td>
<td>Not done</td>
<td>5700</td>
<td>50</td>
</tr>
<tr>
<td>10/M Peptic ulcer</td>
<td>31</td>
<td>78</td>
<td>2.68/70.77</td>
<td>Not done</td>
<td>5800</td>
<td>40</td>
</tr>
<tr>
<td>11/F Menorrhagia</td>
<td>31.5</td>
<td>66</td>
<td>2.14/73.63</td>
<td>Not done</td>
<td>4900</td>
<td>31</td>
</tr>
<tr>
<td>12/M Acute gastritis</td>
<td>27</td>
<td>70</td>
<td>1.25/94.36</td>
<td>Absent</td>
<td>7000</td>
<td>43</td>
</tr>
<tr>
<td>13/F Menorrhagia</td>
<td>32</td>
<td>79</td>
<td>4.46/70.95</td>
<td>Not done</td>
<td>7200</td>
<td>37</td>
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<td>14/M Peptic ulcer</td>
<td>33</td>
<td>80</td>
<td>3.75/73.10</td>
<td>Absent</td>
<td>5700</td>
<td>36</td>
</tr>
<tr>
<td>15/M Peptic ulcer</td>
<td>37</td>
<td>80</td>
<td>3.75/64.34</td>
<td>Absent</td>
<td>6300</td>
<td>30</td>
</tr>
<tr>
<td>16/M Peptic ulcer</td>
<td>33</td>
<td>71</td>
<td>2.14/100.80</td>
<td>Not done</td>
<td>8300</td>
<td>44</td>
</tr>
</tbody>
</table>

*Bone marrow iron stores.
†Colony-stimulating factor, expressed as number of colonies per 50,000 nucleated bone marrow cells (normal range 12–38).
‡Erythropoietin expressed as percent \(^{59}\)Fe incorporation (normal <1%).
iron-deficiency anemia an inverse correlation between packed cell volume (PCV) and CSF, as well as Ep, has been found. These findings suggest that CSF and Ep may have a common cellular or molecular source.

MATERIALS AND METHODS

Sixteen patients with iron-deficiency anemia were studied. The diagnosis of iron-deficiency anemia was based upon a hypochromic, microcytic blood smear, serum iron value of 10.72 μmole/liter or lower, a total iron-binding capacity (TIBC) above 62.61 μmole/liter and a mean corpuscular volume (MCV) of 80 fl or less. Bone marrow biopsy was performed on 8 of 16 patients, and iron stores were absent in all. In this group, patients with chronic or acute infections were specifically excluded. Table 1 summarizes the clinical data in the iron-deficiency anemia patients. Fifteen patients with the anemia of chronic disease were also studied. All of these patients were in a steady state and were without active infections at the time the studies were conducted. The patients were chosen because of an underlying chronic disease with PCV of 22.5–37%,. The diagnosis of anemia of chronic disease was based upon a normal MCV (81–105 fl), serum iron value of less than 10.72 μmole/liter, and TIBC value of 53.61 μmole/liter or less. Bone marrow biopsy was performed in 11 of 15 patients, and all 11 had normal to increased iron stores. Patients in both groups had normal renal function with a creatinine of less than 1.5 mg/dl and a BUN of less than 25 mg/dl. Table 2 summarizes the clinical data of patients with the anemia of chronic disease.

Erythropoietin Assay

The method of Cotes and Bangham was used. Virgin female CF, 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. Five days after removal from the hypobaric chamber, 0.5 ml of serum was injected intraperitoneally into each mouse. On the seventh day, FeCl₃ (0.5 μCi) in 0.5 ml of saline was injected intraperitoneally. The animals were killed on the tenth day. Heparinized blood (0.5 ml) from each animal was placed in a plastic counting vial with an equal volume of heparinized saline and the radioactivity was determined. The ⁵⁹Fe uptake was calculated as a percentage of the total dose with the use of a formula for blood volume as 7% of the body weight, and was used as the measure of the amount of Ep present in the sample. A microhematocrit was determined on each animal, and results in animals with a PCV of less than 60% were discarded from the calculations. Groups of five animals were used for each assay.
Inhibitor Removal

Serum was assayed for CSF level after removal of the lipoprotein inhibitors by the technique described by Granström.28 One volume of serum was mixed with four volumes of chloroform (analytical grade) and shaken for 2 min. The mixture was allowed to stand at room temperature for 1 hr. A thin, surface fat layer was discarded. The second layer of extracted serum was pipetted off and centrifuged at 5000 g for 20 min. The clear supernatant was left at 4°C overnight and then tested for CSF.

CSF Assay

The assay for CSF was done by a modification of the technique of Robinson and Pike, described in detail elsewhere.29 In brief, 0.1 ml of extracted serum was mixed with 1 ml of McCoy's 5A medium with 15% fetal calf serum and 0.3% agar containing 50,000 nucleated human bone marrow cells in 35-mm plastic Petri dishes. Adherent cells were removed from the bone marrow by glass adherence as described by Messner et al.30 Plates were incubated at 37°C with 100% humidity in an atmosphere of 7.5% CO2 in air. The colony counts were done at day 14 with the aid of a dissecting microscope. All experiments were done in triplicate and the mean colony count of the three plates was expressed as the CSF level. All sera were assayed simultaneously using the bone marrow from a single human donor after informed consent and approval from the Human Subject Committee of the University of Colorado Medical Center were obtained. Bone marrow when cultured without added CSF produced less than five colonies per plate.

RESULTS

The serum CSF and Ep values for all patients in the study are shown in Tables 1 and 2. There was no correlation between CSF level and white blood cell counts in either patient group.

Figure 1 shows the correlation between the PCV and serum CSF levels in patients with iron-deficiency anemia. Of the 16 patients with iron-deficiency anemia, 11 had elevated levels of serum CSF (normal range for chloroform-extracted serum 12–38 colonies, mean 28). The mean CSF value in this group was also elevated at 47.3 colonies/50,000 nucleated nonadherent human bone marrow cells (p < 0.005). As noted in Fig. 1, the CSF levels were inversely related to the PCV with a correlation coefficient of 0.74 (p = 0.0005). The mean CSF level in patients with anemia of chronic disease was also elevated at

![Graph](image)
41.9 colonies. However, there was no significant correlation between CSF levels and PCV in patients with anemia of chronic disease.

Of the 16 patients with iron-deficiency anemia, 14 had elevated serum levels of Ep (normal <1% $^{59}$Fe uptake) with an inverse correlation between the PCV and Ep levels. The correlation coefficient was 0.82 ($p < 0.0005$). The mean Ep level in patients with anemia of chronic disease was slightly elevated at 1.51% $^{59}$Fe uptake. There was no significant correlation between Ep levels and PCV in patients with anemia of chronic disease.

Figure 2 shows the correlation between serum Ep and CSF levels in patients with iron-deficiency anemia. A direct relationship was evident with a correlation coefficient of 0.70 ($p = 0.002$). There was no correlation between serum Ep and CSF levels in patients with anemia of chronic disease.

DISCUSSION

These studies have demonstrated that both CSF and Ep levels were elevated in the serum of patients with iron deficiency anemia. Serum levels of Ep correlated inversely with the PCV in patients with iron-deficiency anemia. This observation has been noted previously.26,27 There was no correlation between Ep levels and PCV in patients with anemia of chronic disease, in agreement with previous observations from our laboratory.26,31 Lukens reported a relative failure in production of Ep in rats with experimental arthritis.32 On the other hand, a number of authors have noted elevated levels of Ep in certain patients with anemia of chronic disease.33,35 In the study of Zucker et al. serum Ep levels were elevated only in patients with malignancy in contradistinction to patients with infections or inflammatory diseases.33 It is noteworthy that the present study contained only two patients with malignancy. Half of the chronic disease group of Douglas and Adamson had inappropriately low urinary Ep levels, but they found no difference between Ep excretion in patients with infection or inflammation and those with malignancy.34 It is actually not surprising that data on the anemia of chronic disease were not always consistent since this syndrome involves so many different diseases with so many complicating metabolic features.
The inverse correlation of CSF levels with PCV and their direct correlation with Ep levels in iron-deficiency anemia has not been previously noted. The reasons behind this correlation in human iron-deficiency anemia and in cyclic neutropenia in the grey collie dog are uncertain. Ep and CSF would appear to be under totally separate control mechanisms. The major controlling mechanism for CSF production appears to be bacterial or antigenic load. It is well established that CSF is derived predominantly from the monocyte-macrophage complex. Gordon et al. have proposed that Ep is actually derived from the liver in the form of an inactive precursor which is activated by a renal enzyme. With further recent evidence that the Kupffer cells of the liver are a source of Ep, it is possible that the RES is an important source for both humoral agents regulating hematopoiesis. This thesis may explain the similar behavior of Ep and CSF in patients with iron-deficiency anemia. Alternatively, the similar biochemical properties and behavior of Ep and CSF may be related to their origin from a common precursor molecule. The lack of correlation between Ep and CSF levels in patients with anemia of chronic disease remains to be explained. It has been documented that the anemia of chronic disease involves an abnormality of the RES characterized by impaired release of iron. It is possible that an RES abnormality may also be responsible for impaired Ep production or release.

The reason for normal granulocyte numbers despite elevated serum CSF levels in patients with iron-deficiency anemia is unclear. The responsiveness of the bone marrow of these patients to CSF needs further investigation. Understanding of the exact nature of the relationship between Ep and CSF may have significant bearing on our understanding of normal hematopoiesis and various human disease processes.

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