Inhibition of Human Erythroid Colony-Forming Units by γ Interferon Can Be Corrected by Recombinant Human Erythropoietin

By Robert T. Means Jr and Sanford B. Krantz

Tumor necrosis factor (TNF), interleukin-1 (IL-1), and γ interferon (γIFN) inhibit erythropoiesis in vivo and in vitro, and have been implicated in the pathogenesis of the anemia of chronic disease. Anemia in patients with rheumatoid arthritis and in animals exposed chronically to IL-1 and TNF can be corrected by the administration of recombinant erythropoietin (Epo). We exposed highly purified human erythroid colony-forming units (CFU-E) cultured from peripheral blood burst-forming units-erythroid (BFU-E) and unpurified human marrow CFU-E to recombinant human γIFN and showed inhibition of colony formation in vitro. This inhibition was reversed by increased concentrations of Epo. The mechanisms by which this effect occurs are unknown at present. Epo may cause a downregulation of γIFN receptor expression on CFU-E or, alternatively, γIFN may cause a downregulation of Epo receptor expression. A full understanding of these mechanisms awaits a more complete comprehension of the regulation of erythropoiesis; however, the effect of Epo in vitro is similar to its ability to correct the anemia of chronic disease in vivo.

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INHIBITION OF CFU-E BY γIFN

Table 1. Effect of rhγIFN on CFU-E Colony Formation

<table>
<thead>
<tr>
<th>rhγIFN (U/mL)</th>
<th>Day-8 CFU-E</th>
<th>LDMN Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0 ± 3.6</td>
<td>100.0 ± 4.8</td>
</tr>
<tr>
<td>10</td>
<td>66.5 ± 2.8</td>
<td>71.0 ± 7.1</td>
</tr>
<tr>
<td>100</td>
<td>59.7 ± 2.5</td>
<td>59.9 ± 5.5</td>
</tr>
<tr>
<td>1,000</td>
<td>34.2 ± 2.7</td>
<td>40.4 ± 1.2</td>
</tr>
</tbody>
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Day-8 (highly purified) CFU-E purity in three experiments was 38.1% ± 9.7% (76 ± 19 colonies/clot), LDMN cell purity 0.29% ± 0.03% (58 ± 6 colonies/clot) in three experiments. Results are expressed as mean ± SE.

then enriched for light density mononuclear cells (LDMN cells) by separation over FH.

Culture of CFU-E in plasma clots. CFU-E were cultured at concentrations of 10^5 PB day-8 cells (highly purified CFU-E)/mL or 10^6 LDMN marrow cells/mL with IMDM, 15% FCS, 15% pooled human AB serum, 10% HSA or BSA, rhEpo 1 U/mL, penicillin, streptomycin, epsilon aminocaproic acid 1.5 mmol/L, fibrinogen 1.3 mg/mL (Fibrinogen Kabi, Grade L; Kabi Diagnostics, Stockholm, Sweden) and thrombin 0.2 U/mL (Parke-Davis Pharmaceuticals, Morris Plains, NJ) with varying concentrations of rhγIFN. In some experiments, cells were cultured with rhγIFN 100 U/mL or 1,000 U/mL and varying concentrations of rhEpo. Cells were cultured for 7 days at 37°C in 5% CO₂/95% air and then fixed and stained with benzidine-hematoxylin as described by McLeod et al. Human CFU-E were defined as colonies of 8 to 49 hemoglobinized cells. Each point was studied with three to eight replicates per experiment, and results were normalized to the growth of control plasma clots (cultured without rhγIFN and at an rhEpo concentration of 1 U/mL, expressed as 100%) so that results of different experiments might be compared. The percent CFU-E purity was defined as (number of CFU-E in a clot / number of cells plated in that clot) × 100. PB day-8 cells were cultured at 200 cells per clot and LDMN marrow cells at 0.2 × 10^5 cells per clot. Statistical comparison was by t-test.

RESULTS

Colony formation by highly purified CFU-E (mean purity, 38.1% in three experiments) and unpurified LDMN marrow CFU-E (mean purity, 0.29% in three experiments) was significantly inhibited (P < .001 compared with control) by rhγIFN at all concentrations tested (Table 1). Colonies resistant to the effect of rhγIFN were not morphologically distinguishable from colonies not exposed to rhγIFN.

The effect of increased concentrations of rhEpo on the inhibition of CFU-E colony formation by rhγIFN was initially studied at an rhγIFN concentration of 100 U/mL. Inhibition of highly purified CFU-E (mean purity, 29.2% CFU-E in three experiments) and LDMN marrow CFU-E...
rhEpo can correct the anemia of patients with rheumatoid arthritis as well as the inhibition of erythropoiesis caused by IL-1 or TNF in vivo or in vitro. γIFN, like TNF and IL-1, is associated with inhibition of erythropoiesis in vivo and in vitro; and we have recently reported that inhibition of CFU-E by IL-1 is mediated by γIFN. Our studies indicate that the inhibition of CFU-E colony formation caused by rhγIFN, like that caused by other cytokines, may be corrected by exposure to higher levels of rhEpo.

Mamus et al studied the effect of rhγIFN on CFU-E colony formation from human marrow cells and did not find any correction of inhibition by Epo in cells exposed to rhγIFN. We believe that this finding reflects a difference in experimental design. Mamus et al used much higher concentrations of rhγIFN (3,200 and 10,000 U/1.1 mL) and lower (0.5 to 5.0 U/1.1 mL) concentrations of nonrecombinant Epo. We believe that these differences account for the apparent conflict in our results.

The correction of colony formation by highly purified CFU-E or unpurified marrow CFU-E by higher doses of rhEpo does not reflect a compensatory increase in colony formation by additional CFU-E stimulated with increased rhEpo, because maximum colony formation by these CFU-E is obtained at an rhEpo concentration of 1 U/mL. As our results show (confirming earlier reports from this laboratory), higher levels of rhEpo do not result in significantly increased colony formation from normal CFU-E. Instead, rhEpo appears to be overcoming an inhibitory process induced in individual CFU-E by rhγIFN.

The mechanism by which rhEpo overcomes the inhibitory effect of rhγIFN remains a topic for speculation. rhEpo may downregulate γIFN receptors on CFU-E, thus reducing the response to the inhibitor. Support for this concept by analogy comes from studies indicating that granulocyte-monocyte colony-stimulating factor (GM-CSF) overcomes the inhibitory effect of rhγIFN on human granulocytic progenitors, and that GM-CSF downregulates γIFN receptor expression on monocytes. Alternatively, rhγIFN may downregulate Epo receptor expression on CFU-E, with the CFU-E then requiring higher rhEpo concentrations for optimum colony formation. These possible mechanisms can be tested using highly purified CFU-E, and such studies are presently underway in our laboratory. It is also possible that these inhibitory effects may result from alterations in the Epo-stimulated signal transduction pathway, but clarification of this possibility would have to await a more complete understanding of the mode of action of this growth factor.

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Inhibition of human erythroid colony-forming units by gamma interferon can be corrected by recombinant human erythropoietin [see comments]

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