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Erythropoietin: Current Status

H. FRANKLIN BUNN, M.D.

*Hematology Division, Brigham and Women's Hospital, Harvard Medical School,
Boston, Massachusetts*

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Understanding the regulation of red blood cell production has been greatly enhanced by the cloning and expression of the gene for human erythropoietin (Epo) and its receptor. The availability of recombinant human erythropoietin (rhEpo) for administration to patients has ushered in a new era in molecular medicine. Intravenous or subcutaneous administration of rhEpo can reliably cure the anemia of chronic renal failure and may be effective in the treatment of anemias secondary to chronic inflammation, malignancy, and marrow suppression from chemotherapy. In addition, rhEpo therapy will probably play a prominent role in transfusion medicine, both in preparing patients for auto-transfusions as well as in minimizing red cell transfusion requirements in the post-operative period.

One of the most dramatic successes in the application of modern molecular biology to medicine has been the cloning of the erythropoietin (Epo) gene [1,2] and the high-level expression of recombinant human Epo (rhEpo), enabling its administration to patients. In this brief review, I will first discuss erythropoiesis, *in vitro* and *in vivo*, and its regulation by Epo. This background information is highly relevant to the development of rhEpo for effective treatment of various anemias as well as to other aspects of transfusion medicine.

ERYTHROPOIESIS

Erythroid progenitor cells are direct descendants of trilineage hematopoietic stem cells, as shown in Fig. 1. Early erythroid differentiation is stimulated by at least two hematopoietic growth factors: interleukin 3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF). These lymphokines appear to be liberated by both macrophages and T lymphocytes, probably triggered by cell-cell interactions. Two types of erythroid progenitor cells have been identified by culture in semi-solid media: BFU_e (burst-forming unit, erythroid) and CFU_e (colony-forming unit, erythroid). After eight to ten days in culture, a single BFU_e proliferates into a burst colony, containing up to 2,000 nucleated erythroid cells in discrete subcolonies. The BFU_e is moderately sensitive to erythropoietin but also requires other hematopoietic growth factors, as mentioned above. BFU_e differentiate into CFU_e. The CFU_e can be readily identified after two to three days in culture. A single CFU_e proliferates into a small colony, containing 8 to 64 nucleated erythroid cells. The CFU_e is highly sensitive to erythropoietin. Thus the CFU_e, under the influence of Epo, differentiates into erythroid precursors, readily identified by the accumulation of hemoglobin in the cytoplasm.

The maturation from the earliest recognizable erythroid precursor to the fully developed red cell normally spans four to six days and involves four to five cell divisions.

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Abbreviations: BFU_e: burst-forming unit, erythroid CFU_e: colony-forming unit, erythroid Epo: Erythropoietin GM-CSF: granulocyte-macrophage colony-stimulating factor IL-3: interleukin 3 rhEpo: recombinant human erythropoietin

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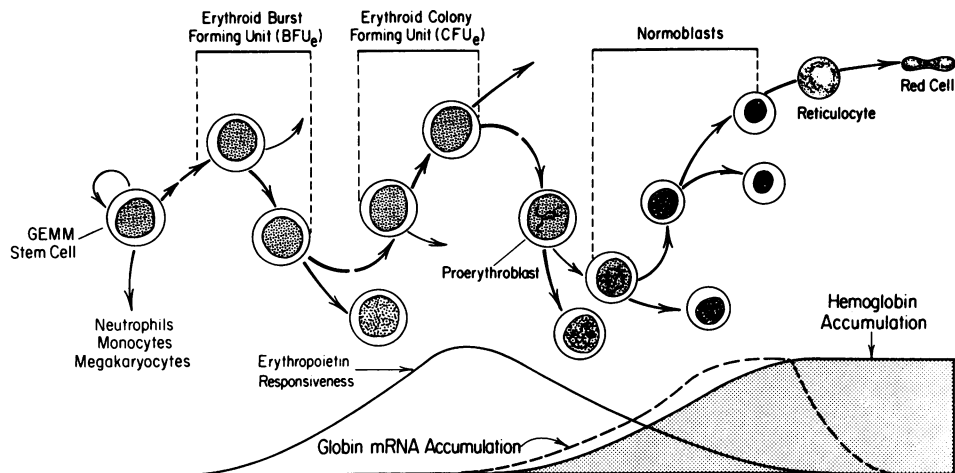


FIG. 1. Differentiation and maturation of erythroid cells. Erythroid progenitor cells are derived from pluripotent stem cells shown on left. Under the influence of Epo, committed erythroid progenitor cells progress through the BFU_e and CFU_e stages into proerythroblasts, the earliest recognizable red cell precursor in the bone marrow. During further maturation, globin mRNA accumulates, directing the cell to produce hemoglobin (from Bunn HF: Pathophysiology of the anemias. In Harrison's Principles of Internal Medicine, 12th edition. Edited by E Braunwald et al. New York, McGraw-Hill, 1990).

The nucleus is shed during the passage from the hematopoietic compartment of the marrow into the vascular sinus. The neonatal red cell (the reticulocyte) becomes much more deformable following the loss of its nucleus.

ERYTHROPOIETIN

This circulating polypeptide hormone is the primary regulator of erythropoiesis in man and other mammals. As shown in Fig. 2, this regulation is based on an elegant "negative feedback" servomechanism, whereby hypoxia is sensed in the kidney, resulting in increased production of Epo by the kidney. Because of an elevated level of Epo in the plasma, marrow erythroid precursor cells (primarily CFU_e) are allowed to proliferate and differentiate, leading to an increase in the red cell mass. If, as expected, this expansion of red cells corrects the hypoxia, Epo production in the kidney will be suppressed.

The human erythropoietin gene encodes a protein of 193 amino acids. Following cleavage of a 27 amino acid N terminal "leader" sequence, the mature protein has a calculated molecular weight of 18,490. Human Epo circulating in plasma lacks its C terminal amino acid, arginine. This residue is probably cleaved by an intracellular carboxypeptidase prior to export from the kidney; it is not known whether this modification has any functional significance. Post-translational glycosylation is clearly required for *in vivo* function. Human Epo contains 39 percent carbohydrate, giving it a molecular weight of 30,400. Complex N-linked glycosylation takes place on three asparagine residues. Human and monkey Epo have one O-linked glycosylation site at residue Serine 126. In addition, both human and monkey Epos contain two disulfide bonds. Both appear to be essential for the function of the molecule. One of the disulfide bonds tethers the N terminal and C terminal ends of the molecule together. The three-dimensional structure of Epo has not yet been determined; however, computer-

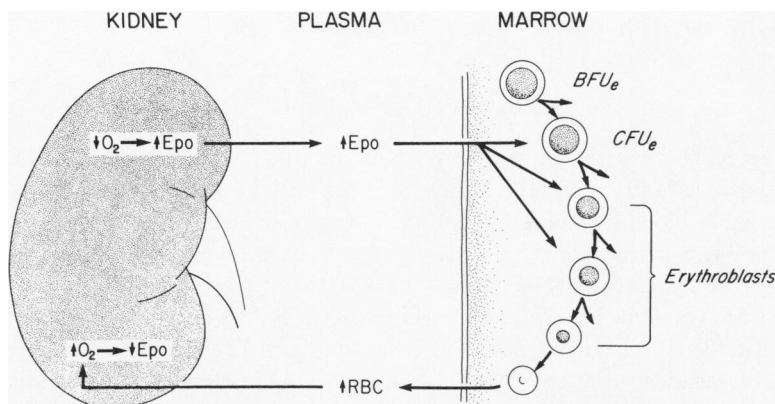


FIG. 2. Regulation of Epo production in the kidney. A hypoxic signal triggers increased production of Epo in the kidney. The increased level of Epo in the plasma stimulates committed erythroid progenitors in the bone marrow to increase red cell production.

based modeling suggests that the molecule probably folds into a globular structure, having two anti-parallel pairs of helical bundles.

The cloning of the Epo gene has permitted large-scale production of human Epo. The carbohydrate structure of the recombinant molecule does not differ significantly from that of native Epo. Moreover, rhEpo has the same disulfide bond structure as native Epo. Indeed, no significant structural differences between the two molecules have been reported. After intravenous infusion, both the native and the recombinant molecules have a half-life in the plasma of about five hours.

The mechanism of action of Epo is not well understood. The hormone binds to a specific receptor, which is expressed only on erythroid precursor cells; this unique 507 residue membrane protein has recently been cloned and expressed [3]. The entire molecule is rich in cysteine residues. The Epo receptor has a single trans-membrane domain. Its cytoplasmic domain is rich in proline residues and lacks any homology to tyrosine kinases; its mRNA is detected only in erythroid precursor cells. The Epo receptor is a member of a recently recognized family of growth factor receptors; it has sequence homology with receptors for granulocyte-macrophage colony-stimulating factor, interleukin 3, interleukin 4, interleukin 6, and with the β subunit of the interleukin 2 receptor. The Epo receptor appears to be oriented as a homodimer on the surface of erythroid cells. Only the subset of receptors with high affinity for Epo has biologic activity.

Very little is known about the molecular events following the binding of Epo to its receptor that lead to proliferation and differentiation of erythroid cells. In addition to stimulating division of CFU_e , thereby increasing production of erythroblasts, erythropoietin also hastens the rate at which erythroid cell divisions occur and lowers the barrier between marrow and blood that normally retains young reticulocytes in the marrow compartment [4]. The binding of Epo to its receptor results in both the proliferation and the terminal maturation of erythroid cells, with induction of biosynthesis of erythroid-specific proteins such as hemoglobin and certain red cell membrane proteins (Fig. 1). There is growing evidence that the Epo-responsive precursor cell is already destined to differentiate into erythroid cells and that Epo salvages these

dividing cells from programmed death, thereby allowing expansion of red cell production [5].

EPO PRODUCTION

In the fetus, the liver is the primary source of Epo. After birth, the kidney is the major site of production [6]; the kidney is a logical site because it would sense the redistribution of blood flow in mild anemia. The precise localization of Epo production in the kidney is controversial; the best evidence to date favors a subset of peritubular interstitial (endothelial) cells in the boundary between the inner cortex and the outer medulla [7,8]. The liver is normally a less important source of Epo, but it can be called upon in patients with impaired or absent renal function. The level of Epo mRNA in the kidney increases by at least twentyfold when an animal is subjected to acute hypoxia or treated with cobalt chloride, an agent known to induce erythrocytosis in experimental animals in association with increased levels of plasma Epo.

Epo is produced in a regulated fashion in a human hepatoma (liver carcinoma) cell line (Hep3B) [9]. In these cells, both hypoxia and cobalt chloride trigger a 50- to 100-fold increase in Epo mRNA. Recent studies in Hep3B cells suggest that the oxygen sensor for erythropoietin regulation is a heme protein. A likely mechanism whereby cobalt induces Epo production is by substituting for iron in the heme group of the sensor protein, thereby mimicking the deoxy state [9]. When Hep3B cells are challenged with either hypoxia or cobalt, there is both increased transcription of the Epo gene and enhanced stability of the Epo mRNA.

THERAPY OF ANEMIA OF UREMIA WITH rhEpo

In patients with uremia the severity of anemia is roughly related to the degree of renal failure. Although some patients tolerate their anemia fairly well, it is often a major cause of limitation in activity and even of disability. Plasma Epo is lower in uremic patients compared to levels in patients with other types of anemia of comparable severity.

A number of clinical trials have shown that the intravenous administration of recombinant human Epo three times weekly will enable most patients to achieve and maintain a normal red cell mass [10-12]. As shown in Fig. 3, initial treatment is associated with reticulocytosis and an increase in red cell utilization of iron, reflecting effective erythropoiesis [10]. This treatment is expected to relieve the secondary iron overload that is commonly encountered in renal dialysis patients, particularly if they have been transfused. Too rapid restoration of red cell mass can cause significant hypertension; however, no other significant adverse effects have been noted either in uremic patients or in those with other types of anemias. A theoretical pitfall is the development of antibodies to rhEpo owing to possible subtle differences in carbohydrate structure between the native and recombinant molecules. Among over 1,000 patients who have been treated with rhEpo thus far, however, none have become refractory to therapy owing to the development of antibodies. The treatment of hemodialysis patients with rhEpo has been extremely gratifying. A large proportion have reported a striking improvement in their performance status and overall quality of life [13]. Uremic patients who are not yet dependent on dialysis have also benefited from rhEpo therapy without any detectable effect in the rate of deterioration of renal function [14]. Recently, the subcutaneous administration of rhEpo has been shown to

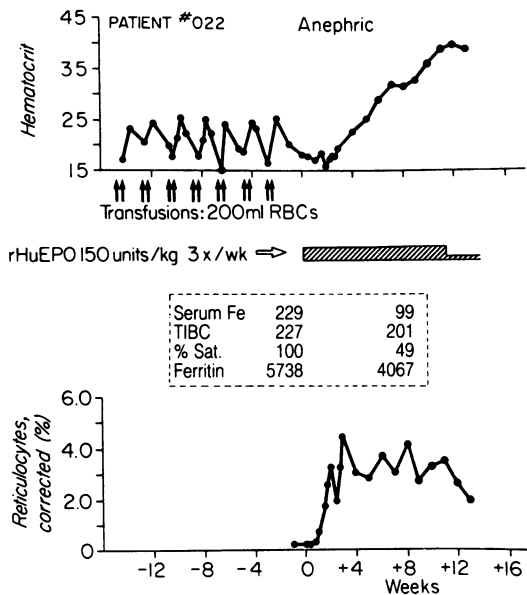


FIG. 3. Response of a patient with renal failure on hemodialysis to the administration of recombinant human Epo (rHuEpo). The same time scale (weeks) applies to both the upper and lower panels. Prior to Epo therapy, the patient required frequent blood transfusions (vertical arrows). The top panel shows the hematocrit, while the bottom panel shows the concomitant percentage of reticulocytes over a 26-week period. The box insert shows changes in serum iron (Fe), total iron binding capacity (TIBC), percentage of iron saturation (% Sat.), and serum ferritin. Note that Epo therapy resulted in reticulocytosis and subsequent increase in hematocrit, with cessation of requirement for blood transfusions. The increase in red cell mass was associated with a significant depletion of body iron stores (from [9]).

be both safe and effective. The peak plasma levels of Epo are much lower than those following intravenous therapy, but the delivery of the rhEpo is more sustained.

OTHER APPLICATIONS OF rhEpo

Unlike the anemia of uremia, other types of anemia are associated with an exponential increase in the level of plasma Epo in proportion to the decrease in red cell mass [15]. Patients with high plasma Epo levels might not be expected to respond to rhEpo therapy. Nevertheless, recent clinical trials have demonstrated a significant improvement in hematocrit in patients with the anemia of chronic inflammation (particularly patients with rheumatoid arthritis) [16] and with the anemia of malignancy. In addition, rhEpo will probably prove to be useful in hastening the recovery of erythropoiesis following marrow transplantation or chemotherapy. In particular, rhEpo has proven to be effective in AIDS patients with myelosuppression secondary to treatment with azidothymidine and in patients with testicular cancer who have been treated with cis-platinum. Administration of rhEpo has been proposed as a means of increasing the level of fetal hemoglobin in patients with sickle-cell anemia, thereby impairing the polymerization of Hb S. Unfortunately, we have not observed any significant increase in Hb F in five SS patients who have been treated with high doses of rhEpo.

Of particular relevance to transfusion medicine is use of rhEpo in the preparation of patients for elective surgery, enhancing the number of units that can safely be removed and preserved for autologous transfusion in the peri-operative and post-operative periods. Well-designed studies both in primates [17] and in man [18] indicate that this strategy is safe and effective. Moreover the administration of rhEpo may be useful in decreasing the amount of red cell transfusions required in the post-operative period. Finally, rhEpo may be useful in enhancing the amount of blood that can be removed

from donors. It is likely that rhEpo treatment would enable a reduction in the minimal interval (currently eight weeks) between repeat phlebotomies. This treatment might facilitate a recipient's receiving multiple transfusions of blood from the same donor and therefore a reduction in the incidence of transfusion-transmitted diseases.

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