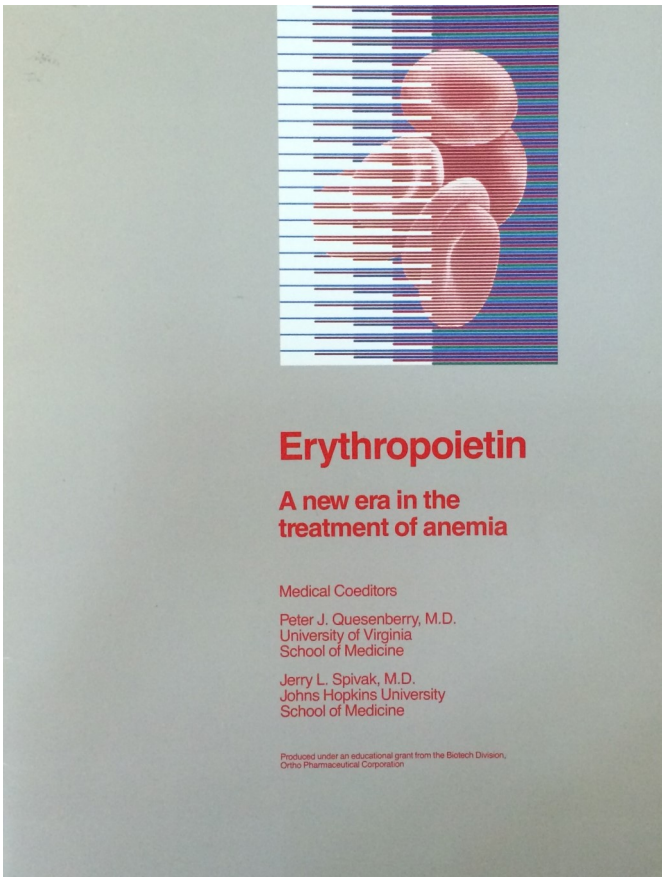
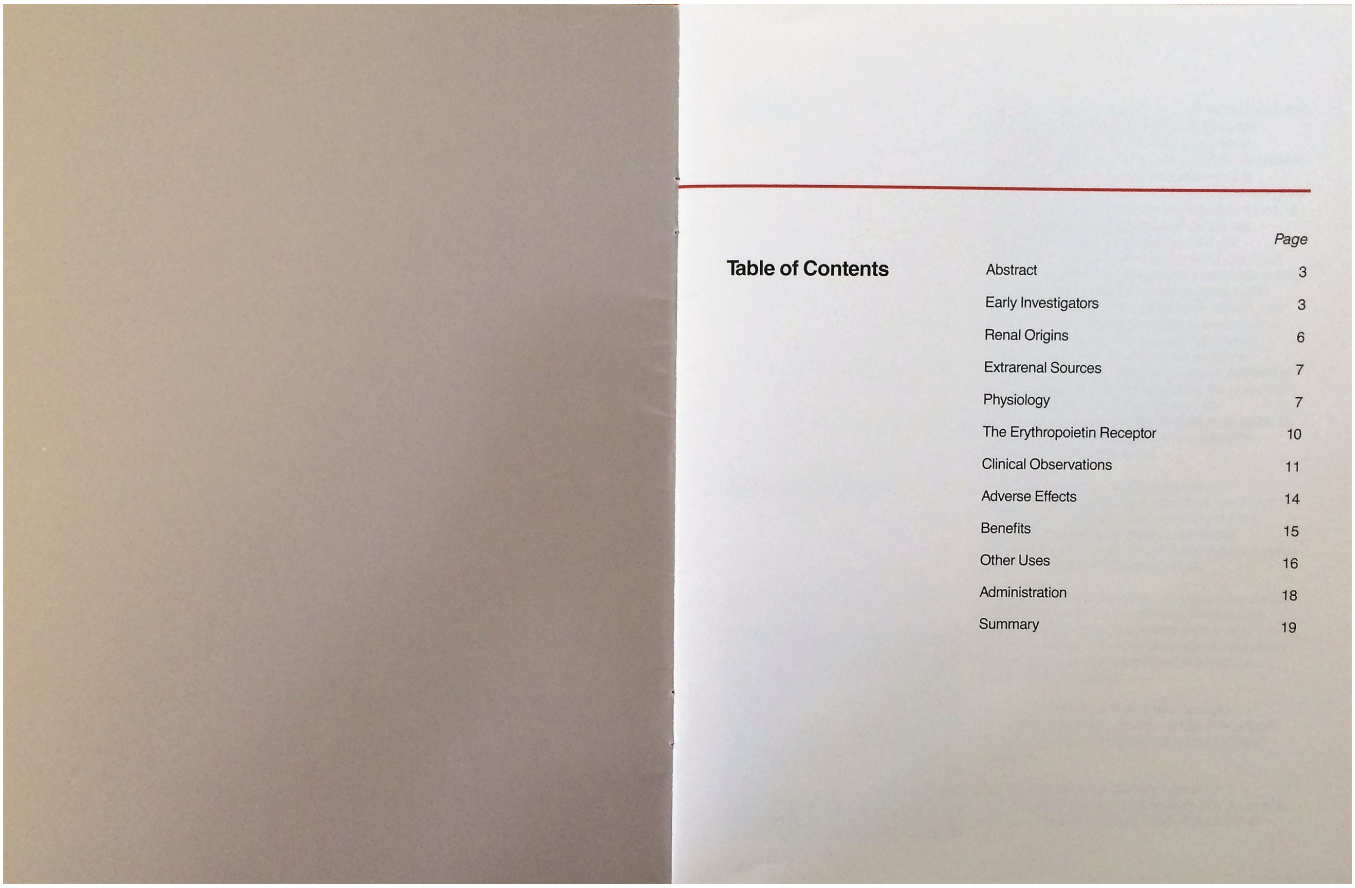


ORTHO BIOTECH
Erythropoietin Introduction



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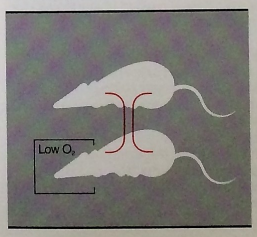
Abstract

Erythropoietin is a glycoprotein hormone, produced primarily by the kidneys, that stimulates the proliferation and differentiation of erythroid progenitor cells. Normally, erythropoietin is present in the plasma at a low concentration that is sufficient for the replacement of red blood cells lost to aging. Situations such as anemia, blood loss, or hypoxia trigger the production of additional erythropoietin, which accelerates red cell production. The gene for the hormone has been cloned and expressed in cultured mammalian cells to produce recombinant human erythropoietin (r-HuEPO) in quantities sufficient for clinical use. Studies are currently in progress to evaluate the effect of r-HuEPO on the anemia associated with disorders such as chronic renal failure, rheumatoid arthritis, AIDS, and cancer. Additional investigational studies are exploring the potential of r-HuEPO as a means of coping with intraoperative blood loss by facilitating donation for autologous transfusion, and as an adjunct to therapy of sickle cell anemia.

Early Investigators

The first suggestion that the regulation of erythrocyte production has a humoral mechanism dates back to 1906 and the University of Paris. During the following 50 years, substantial experimental evidence accumulated supporting the humoral regulation of erythropoiesis.

Fig 1.—Erythropoiesis increases in a rat parabiotically joined to another in a hypobaric environment.

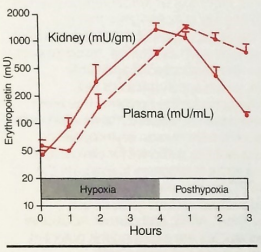


The definitive demonstration of humoral regulation of erythropoiesis was furnished by Reissmann (1950), who observed increased erythrocytic activity in the bone marrow of parabiotic rats, even though only one was maintained in a hypoxic environment (Figure 1).

Erslev (1953) was the first to demonstrate that plasma from severely anemic rabbits (hematocrit 20%) could induce a significant reticulocytosis in rabbits.

That the kidney is the primary source of erythropoietin was first demonstrated by Jacobson and his colleagues (1957). They observed that

Fig 2.—Plasma and kidney erythropoietin content in rats after onset of hypoxia and during posthypoxic period.



plasma erythropoietin was significantly reduced in anemic animals after bilateral nephrectomy, but not after bilateral ureteral ligation. Further, Jelkman (1986) and Caro et al (1981) showed that, in response to hypoxia, the erythropoietin concentration in renal tissue rises before an increase is seen in the circulation (Figure 2). Most recently, by in situ hybridization studies of erythropoietin mRNA expression, several groups of investigators have identified peritubular interstitial cells as the site of renal erythropoietin synthesis (Koury et al, 1988; Lacombe et al, 1988).

Erythropoietin was first purified from human urine in 1977 by Miyake et al. This process required over 2,500 liters of urine from severely anemic patients and yielded only a few micrograms of the hormone. Pure human urine erythropoietin is a heavily glycosylated protein with a molecular weight of 30,400 daltons, of which 39% is carbohydrate (Figures 3 and 4).

In 1984, Eschbach et al provided convincing evidence of the therapeutic potential of erythropoietin. Sheep made uremic (and, therefore, anemic) by surgical ablation of the kidneys were maintained on hemodialysis. Plasma containing high concentrations of erythropoietin was harvested from anemic sheep with normal renal function. When this plasma was infused into the anemic sheep, there was an increase in erythropoiesis and correction of the anemia. But there was still no adequate source of highly purified erythropoietin for clinical use in humans.

In 1985, molecular biologists Jacobs and Lin each were able to sequence and clone the gene for human erythropoietin. Subsequent bulk cultures of mammalian cells into which the gene had been inserted provided sufficient recombinant human erythropoietin (r-HuEPO) for clinical trials. In both in vitro and in vivo assays, the activity profiles of r-HuEPO and urinary erythropoietin are identical (Browne et al, 1986), as is the chemical composition of the two types of erythropoietin (Recny et al, 1987).

Fig 3.—Biochemistry of erythropoietin.

- MW 30,400 daltons
- Single chain polypeptide with 2 internal sulfhydryl bonds
- Carbohydrates constitute 39% of its mass
- Erythropoietin is very hydrophobic
- Human urine and recombinant erythropoietin are identical in composition

Fig 4.—Genetically engineered recombinant human erythropoietin.

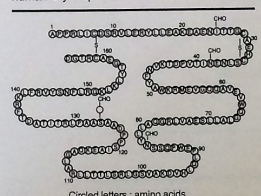


Table 1—Time Line

1906	Serum of anemic rabbits contains erythropoietic factor	Carnot & Deflandre
1950	Hypoxia causes increased erythrocytic activity in bone marrow	Reissmann
1957	Nephrectomy lowers erythropoietin titer	Jacobson et al
1977	Extracts and purifies erythropoietin from urine of anemic humans	Miyake et al
1984	Plasma from normal sheep corrects anemia due to nephrectomy	Eschbach et al
	Erythropoietin binding to membrane receptor of erythroid progenitor cells	Krantz & Goldwasser
1985	Sequence and clone gene for human erythropoietin	Jacobs & Lin
1986	Show recombinant human erythropoietin to have activity identical to erythropoietin extracted from urine	Browne et al
	Recombinant human erythropoietin available for clinical study	
1986-1987	r-HuEPO corrects anemia in dialysis patients, reduces need for transfusion	Eschbach et al Winearls et al
1988	Reduced erythropoietin response in rheumatoid arthritis	Hochberg et al
	Virtual elimination of transfusion dependency in uremic patients	Eschbach et al
1989	Preliminary studies indicate improved erythropoiesis in some AIDS patients on AZT	

Renal Origins

The kidneys are the primary site of the blood oxygen sensor and erythropoietin synthesis. This is physiologically appropriate, since they filter 180 liters or more of fluid from plasma daily, but extract little oxygen on their own. Based on organ weight, the rate of blood flow through the kidneys is substantially greater than that through other well-perfused organs such as the heart, brain, and liver. In addition, as pointed out by Erslev and Caro (1986), the kidneys' consumption of oxygen (which is utilized in the tubular reabsorption of sodium) is directly proportional to blood flow (Figure 5). It is possible, therefore, that the compensatory flow rate associated with anemia could create a local hypoxia in the kidney, thus locally amplifying the signal of general hypoxemia, which triggers the synthesis and secretion of erythropoietin (Figure 6).

By in situ hybridization studies, peritubular interstitial cells of the outer renal cortex and medulla, areas of the kidney with a rich blood supply, have been identified as the site of erythropoietin production (Koury et al, 1988; Lacombe et al, 1988).

Fig 5. — Kidney O_2 consumption and blood flow — the relation in the kidney.

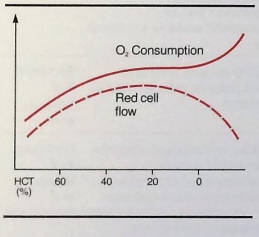
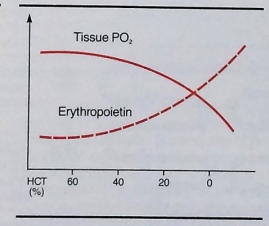


Fig 6. — Kidney tissue O_2 vs erythropoietin production — the relation in the kidney.



Extrarenal Sources

Clinical and experimental observations on anephric humans and animals clearly indicate that the kidney is not the sole source of erythropoietin. About 10% to 15% of erythropoietin is made extrarenally, with most, if not all, of this amount being produced by the liver (Bondurant et al, 1986).

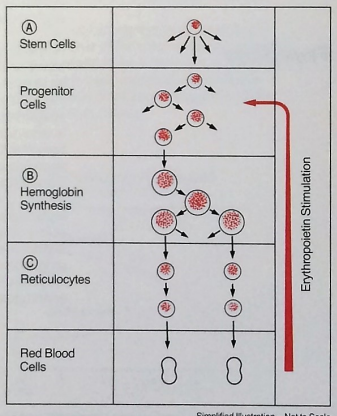
Investigators (Lucarelli et al, 1968; Zanjani et al, 1981) studying fetal lambs and rats have shown that during fetal life, erythropoietin is synthesized primarily by the liver, with a liver-to-kidney switch occurring within the first weeks after birth. Whether this is also true in other mammalian species is unknown. Inappropriate ectopic production of erythropoietin has been observed with a variety of benign and malignant tumors. They include hypernephroma, hepatoma, uterine leiomyoma, and cerebellar hemangioma.

Physiology

Normally, in the adult, the bone marrow is the only site of erythropoiesis. Mature red cells are the product of erythroid progenitor cells, which themselves arise from primitive multipotent progenitor hematopoietic cells. On the basis of in vitro clonal assays, several different classes of committed marrow erythroid progenitor cells have been identified. The most primitive progenitor that responds to erythropoietin is called the burst-forming unit-erythroid (BFU-E) because it produces multiclustered colonies of hemoglobin-synthesizing cells in response to high concentrations of erythropoietin. The BFU-E is the earliest erythroid progenitor cell identified that is erythropoietin-responsive, but it is not completely erythropoietin-dependent.

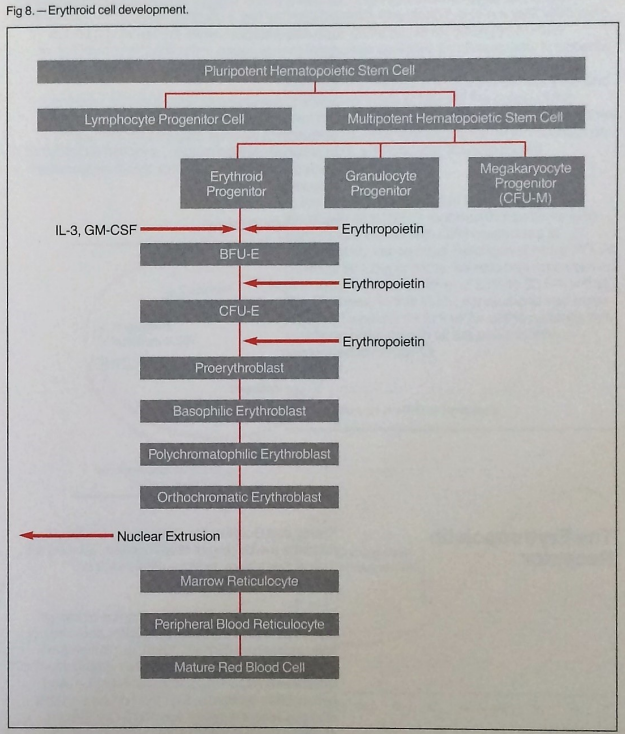
The more mature, colony-forming unit-erythroid (CFU-E), which has a lower proliferative potential than the BFU-E, is absolutely dependent on erythropoietin for its survival, its ability to proliferate, and its ability to differentiate. As differentiation proceeds, erythropoietin dependence declines, a process associated with loss of erythropoietin receptors. The major steps in erythroid differentiation are outlined in Figures 7 and 8.

Fig 7. — Stages of red blood cell development. Schematic representation of the differentiation, maturation, and proliferation of stem cells, erythroid progenitor cells, and erythroid precursor cells under the influence of erythropoietin.



Simplified Illustration — Not to Scale

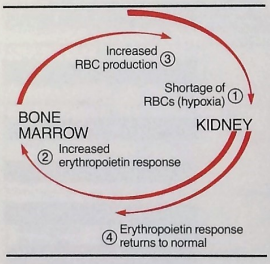
Fig 8. — Erythroid cell development.



The maturation time for a red cell is approximately 7 days. The first 4 days are spent in cell proliferation and differentiation, while the remaining time is spent in maturation, including the completion of hemoglobin synthesis and nuclear extrusion.

In effect, the erythropoietin-erythroid progenitor cell interaction resembles a feedback loop, the operation of which is triggered by a reduction in erythrocyte-carried oxygen, and then suppressed upon restoration of adequate tissue oxygenation (Figure 9).

Fig 9. — The feedback loop.



The Erythropoietin Receptor

Krantz and Goldwasser (1984) were the first to describe the binding of erythropoietin, labeled with radioactive tritium, to high-affinity sites on erythroid progenitor cells.

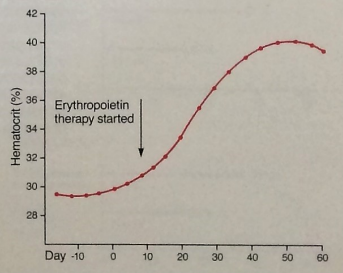
Intracellular events following receptor-binding of erythropoietin to erythroid progenitor cells are not fully understood (Spivak, 1986), but the earliest effect is a rapid stimulation of RNA synthesis, which is followed by DNA synthesis, cell division, and hemoglobin synthesis. Erythroid precursor cells also possess a receptor specific for transferrin, enabling them to incorporate sufficient iron for hemoglobin synthesis.

Clinical Observations

It was not until 1985 that the gene for human erythropoietin was cloned, and not until 1986 were adequate quantities of recombinant human erythropoietin available for clinical trials in patients with anemia due to chronic renal failure (CRF). Although a complete summary of clinical tests and patient responses is beyond the scope of this monograph, Figure 10 (Schwartz et al, 1988) shows the effect of recombinant human erythropoietin on hematocrit, and a review of some recent developments follows.

In the first published clinical trial of r-HuEPO, Eschbach et al (1987) reported that patients with anemia associated with CRF responded to thrice-weekly intravenous injections of r-HuEPO. At doses of 50 U/kg or more, hematocrits rose from an average pretreatment level of 23% to 33.5% within 8 to 12 weeks. In this study, transfusions had been required by nearly three fourths of the patients; this need was eliminated in all the patients who responded to r-HuEPO.

Fig 10. — Effect of r-HuEPO on hematocrit.



During the past 3 years, many clinical studies have confirmed that in anemic patients with CRF, r-HuEPO produces a dose-related elevation of hematocrit, hemoglobin, circulating reticulocytes, and a concurrent alleviation of symptoms of anemia.

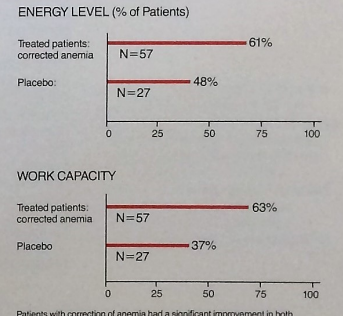
Patients in these studies have reported significant relief of fatigue, listlessness, lethargy, and the symptoms of Raynaud's phenomenon. They also reported improved appetite, better sleep/wake patterns, less depression, and enhanced sexual interest and ability.

Most recently, the ability of r-HuEPO to increase work capacity and energy levels was quantitatively evaluated in double-blind, placebo-controlled trials of the safety and efficacy of r-HuEPO in predialysis patients whose hematocrits were 30% or less. In patients whose kidney failure had progressed to the point of causing anemia, but not so far as to require dialysis, r-HuEPO relieved anemia without worsening renal function. When administered three times a week for 8 weeks, r-HuEPO raised the hematocrit of patients in a dose-related manner. By the criterion of an increase of 6 or more hematocrit points, 27 of 30 patients (90%) responded to 150 U/kg, 22 of 28 (79%) responded to 100 U/kg, and 16 of 30 (57%) responded to 50 U/kg. Recombinant human erythropoietin corrected anemia (attainment of hematocrit of 40% in males and 35% in females) in 87% (26/30) of the patients receiving 150 U/kg, 64% (18/28) of those receiving 100 U/kg, and 46% (13/28) of those receiving 50 U/kg. Only 1 of the 30 patients (3%) receiving placebo had correction of anemia.

The correction of anemia was strongly associated with significant improvement in energy level and work capacity as scored (on a scale of 1 to 5) by the patients. Sixty-one percent of the 57 patients in whom r-HuEPO produced a corrected anemia rated their energy level as significantly increased in comparison to their pretreatment condition. This difference was statistically significant ($P<0.05$), as was the impact of r-HuEPO-corrected hematocrit on the patients' work capacity. Among the patients with corrected anemia, 63% reported improvement in work capacity as compared to 37% reporting some improvement despite a hematocrit that was either not, or only partially, corrected (Figure 11).

In sum, this study showed that r-HuEPO raised hematocrit and increased work capacity and energy levels of predialysis patients while neither increasing serum creatinine nor depressing creatinine clearance, as compared with placebo-treated control patients.

Fig 11. — Erythropoietin improves quality of life.



Patients with correction of anemia had a significant improvement in both parameters ($P<0.05$)

G86-011: 8-Week Study

Adverse Effects

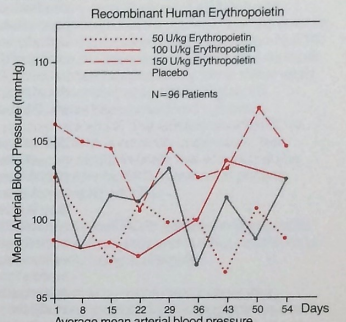
In hemodialysis patients treated with r-HuEPO, blood pressure has tended to rise with the rise in hematocrit, but this was usually controllable with antihypertensive medication (Figure 12). There have also been a few instances of seizures associated with a rise in blood pressure. The cause of hypertension in r-HuEPO-treated patients is not fully understood. It could be due to the correction of vasodilatation associated with anemia and/or to an increased blood viscosity resulting from the increased red cell mass. Both of these conditions, by increasing total peripheral resistance, could cause hypertension. Elevations of blood pressure have also occurred in predialysis patients, but it has been observed that these were more likely to occur if the rate at which the hematocrit increased exceeded 0.2 hematocrit points per day. This suggests that blood pressure increases may be minimized by adjusting the dose of r-HuEPO to keep the rate of hematocrit rise below this level. An excessive increase in hematocrit is also a risk factor for elevation of blood pressure, and this too may be minimized by dosage adjustment.

Iron deficiency requiring iron supplementation can also occur. This is due to insufficient stored iron to permit expansion of the red cell mass.

Other adverse effects that have been seen in dialysis patients, and which may be related to r-HuEPO therapy, include a rise in the predialysis concentration of serum creatinine, BUN, and potassium, along with occasional thromboses at vascular access sites. In patients not yet requiring dialysis, measures of renal function have not differed between placebo and r-HuEPO-treated groups.

No evidence of organ dysfunction, toxicity, or antibody formation has been reported with r-HuEPO therapy.

Fig 12. — Effect of r-HuEPO on blood pressure.



Average mean arterial blood pressure versus time on therapy

Benefits

Clinicians with experience using r-HuEPO take the position that adverse effects detected so far are minimal, compared with the benefits r-HuEPO brings to the treatment of the severe anemia associated with end-stage renal failure. Not the least of these benefits is the elimination of transfusions for most patients. Fewer transfusions mean a reduced risk of iron overload, of exposure to infectious agents, and of the development of antibodies that could threaten the success of renal transplants.

Other Uses

Encouraged by demonstrations that r-HuEPO can alleviate anemia due to erythropoietin insufficiency, investigators throughout the world have initiated trials of r-HuEPO to examine its ability to correct or prevent anemias in a wide variety of diseases in which erythropoietin is deficient. These include anemia due to AIDS, rheumatoid arthritis, cancer, cancer chemotherapy, and intraoperative blood loss.

With respect to blood loss during surgery, preliminary studies indicate that r-HuEPO treatment may permit the predonation of blood for autologous transfusion in greater amounts or in a shorter time than is currently possible. In a recent placebo-controlled study, it has been shown that the amount of blood available for autologous transfusion could be increased by about 25% in response to the combined stimulation of erythropoiesis by r-HuEPO and repeated phlebotomy. Over the course of this study, the r-HuEPO-treated patients donated a mean total of 5.4 units of blood in comparison to 4.1 units donated by the placebo-treated patients. This difference was attributable to the fact that the hematocrit of the placebo-treated patients more frequently fell below 34%, thus disqualifying donation at that time.

A further measure demonstrated that administration of r-HuEPO at the times of donation was able to effectively reduce the decline in hematocrit associated with the serial donation of 1 unit of blood every 3 to 4 days. Despite the fact that the r-HuEPO-treated patients donated significantly more blood, their mean hematocrit immediately before surgery was significantly higher than that in the placebo-treated group.

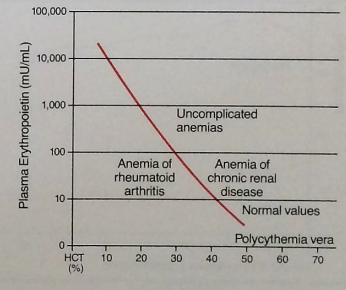
The anemia that develops in the vast majority of AIDS patients is severely exaggerated by treatment with AZT (zidovudine) to the point of requiring transfusions and/or discontinuing AZT therapy. Preliminary results of ongoing r-HuEPO trials in these patients are showing that transfusion dependency can be significantly reduced, suggesting that this effect may have the additional benefit of permitting continued treatment with optimal doses of AZT.

Based on anemia related to a blunted endogenous erythropoietin response in patients with inflammatory complications, Eschbach and Adamson (1988b) have suggested that the anemia of chronic inflammation might respond to r-HuEPO therapy. On the basis that observed erythropoietin levels are often inappropriately low in rheumatoid arthritis (Hochberg et al, 1988) (Figure 13), r-HuEPO has been considered a possible replacement agent. The efficacy of r-HuEPO in such conditions is currently under study, and preliminary results indicate that anemia can be corrected with r-HuEPO treatment in patients with rheumatoid arthritis.

The value of r-HuEPO in restoring erythropoiesis following chemotherapy and in other conditions is also currently being investigated.

Studies done in baboons demonstrate that r-HuEPO can increase fetal hemoglobin production, an effect that could ameliorate the symptoms of sickle cell disease. Whether this can translate into a benefit for sickle cell patients is under investigation.

Fig 13. — Erythropoietin levels in disease states.



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