RECOMBINANT ERYTHROPOIETIN FOR THE TREATMENT OF ANEMIA IN INFLAMMATORY BOWEL DISEASE

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Abstract *Background.* Some patients with inflammatory bowel disease have anemia that is refractory to treatment with iron and vitamins. We examined whether administering iron and recombinant erythropoietin could raise hemoglobin levels in such patients.

Methods. Thirty-four patients with inflammatory bowel disease (15 with ulcerative colitis and 19 with Crohn's disease) and anemia refractory to iron therapy (hemoglobin concentrations below 10.0 g per deciliter [6.2 mmol per liter]) were randomly assigned in a prospective, double-blind, 12-week trial to receive either oral iron (100 mg per day) and subcutaneous erythropoietin (150 U per kilogram of body weight twice per week) (n = 17) or oral iron and placebo (n = 17). The primary measure of efficacy was an increase in hemoglobin levels of more than 1.0 g per deciliter (0.62 mmol per liter). Additional analyses were performed with other patients with inflammatory bowel disease.

Results. The severity of anemia was related to clinical disease activity as well as to in vitro monocyte secretion of interleukin-1β, a proinflammatory cytokine. Serum erythropoietin concentrations were increased in 52 randomly selected outpatients with inflammatory bowel disease and anemia, but the concentrations were inadequate in relation to the degree of anemia. Twelve weeks of therapy with recombinant erythropoietin and oral iron increased mean ($\pm$SE) hemoglobin concentrations from 8.81$\pm$0.27 g per deciliter (5.47$\pm$0.17 mmol per liter) to 10.52$\pm$0.41 g per deciliter (6.5$\pm$0.25 mmol per liter), whereas hemoglobin concentrations in the placebo group decreased from 8.69$\pm$0.11 g per deciliter (5.4$\pm$0.068 mmol per liter) to 7.84$\pm$0.33 g per deciliter (4.9$\pm$0.2 mmol per liter) ($P<0.001$). After 12 weeks, hemoglobin levels had increased by more than 1.0 g per deciliter in 82 percent of the patients in the erythropoietin group, as compared with 24 percent of those in the placebo group ($P=0.002$). There were five treatment failures in the placebo group and two in the erythropoietin group ($P=0.18$); treatment failure was defined as a decrease in hemoglobin levels of more than 2.0 g per deciliter (1.24 mmol per liter) to a value below 8.0 g per deciliter (4.96 mmol per liter) or any decrease to less than 6.5 g per deciliter (4.03 mmol per liter).

Conclusions. In patients with inflammatory bowel disease and anemia refractory to treatment with iron and vitamins, treatment with oral iron and recombinant erythropoietin can raise hemoglobin levels. (N Engl J Med 1996; 334:619-23.)

SOME patients with inflammatory bowel disease have anemia that is refractory to treatment with iron and vitamins. A lack of erythropoietin may be important in the pathophysiology of anemia associated with inflammation,[1,2] if deficiencies in iron, cobalamin, and folic acid have been ruled out.

Erythropoietin is the primary growth factor regulating the proliferation and maturation of erythroid precursor cells.[3] Proinflammatory cytokines, including interleukin-1β, tumor necrosis factor α, and interferon γ, are produced in increased amounts by peripheral-blood monocytes and mononuclear cells of the intestinal lamina propria in patients with inflammatory bowel disease.[4-7] Such cytokines can contribute to the development of anemia by inducing a relative deficiency of erythropoietin as well as a resistance to erythropoietin on the part of erythroid precursor cells in various in vitro and in vivo models.[8-11] In these models, administering erythropoietin could reverse most of the suppression related to proinflammatory cytokines of cell maturation of the erythroid lineage.[8-10] The use of recombinant erythropoietin (epoetin) to treat anemia in chronic rheumatoid arthritis as well as anemia associated with malignant disease has been suggested.[8,11]

We studied patients with inflammatory bowel disease who had chronic anemia refractory to oral iron therapy and evaluated treatment with erythropoietin in a double-blind, prospective, randomized trial.

METHODS

The prevalence of anemia in patients with inflammatory bowel disease who were referred to the University of Hamburg was analyzed between January 1990 and December 1992 with regard to disease characteristics, the secretion of proinflammatory cytokines, and erythropoietin levels. Patients with "treatment-refractory anemia," as assessed by their physicians, were selected. Sustained nonresponsiveness to iron was verified in a six-week preliminary phase. Eligible patients whose anemia was refractory to orally administered iron were randomly assigned to receive subcutaneous injections of recombinant erythropoietin or placebo during a 12-week trial. Written informed consent was obtained from each patient. The trial was reviewed by the ethics committee of the University of Hamburg.

Preliminary Phase and Randomization

Patients with confirmed diagnoses of Crohn's disease or ulcerative colitis who had refractory anemia were referred for possible erythropoietin treatment. Criteria for exclusion from the study were the use of immunosuppressive agents during the previous four months, arterial hypertension resistant to therapy, pregnancy or lactation, impaired renal function (serum creatinine levels above 1.2 mg per deciliter [106.1 μmol per liter]), obvious need for surgery (as indicated by the severity of disease and of complications), and anemia from other causes (abnormal erythrocyte morphology or severe iron deficiency [e.g., due to intestinal blood loss or associated with ferritin levels of $<$20 ng per milliliter in women and $<$30 ng per milliliter in men]). Vitamin deficiency was ruled out by examination of the erythroid morphology and assessment of serum levels of folic acid and cobalamin. A medically approved plan of contraception was required for all menstruating women. Patients with a history of anemia and hemoglo-

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bin levels below 10.0 g per deciliter (6.2 mmol per liter) were enrolled in a six-week preliminary phase in which they received oral iron (at least 100 mg per day). Patients were seen at least once a week.

Only the 34 patients (15 with ulcerative colitis and 19 with Crohn's disease) with hemoglobin levels of ≤10.0 g per deciliter (6.2 mmol per liter) throughout the preliminary phase were randomized. Seventeen received erythropoietin (150 U per kilogram of body weight administered subcutaneously twice a week [Erypo, Cilag, Sulzbach, Germany]), and 17 received placebo (Table 1). Concomitant treatment with iron (100 mg per day [Ferrosanol-duodenal, Sanol, Monheim, Germany]) was required. Antiinflammatory treatment (with corticosteroids or aminosalicylates) was administered according to the severity and site of disease. Groups were similar in age, sex, duration of disease, disease manifestation, duration of present remission, antiinflammatory regimens, and previous treatment. The original trial design included a crossover into a second 12-week treatment period. However, because of a strong carryover effect (most of the patients in the erythropoietin group had already responded), the second period was dropped.

Study Protocol

At the initial visit, a medical history was taken for each patient and a physical examination and stool examination for ova, parasites, and enteric pathogens were carried out. Blood was drawn for the assessment of electrolytes, protein, albumin, glucose, liver enzymes, amylase, creatinine, urea, erythropoietin, serum iron, ferritin, and in vitro monocyte secretion of interleukin-1β, and to determine the differential blood count. The physical examination and routine laboratory assessment were repeated weekly. After six weeks, the serum concentrations of erythropoietin, iron, and ferritin were assessed and the studies from the initial visit repeated.

Table 1. Base-Line Characteristics and Conditions of the 34 Patients.

Characteristic or Condition	Erythropoietin Group (N = 17)	Placebo Group (N = 17)
Ulcerative colitis (no. of patients)	8	7
Pancolitis	6	4
Left-sided colitis	1	1
Clinical Colitis Activity Index		
Median	5.5	6
Range	3–10	4–11
Crohn's disease (no. of patients)	9	10
Ileal	9	9
Colonic	9	10
Crohn's Disease Activity Index		
Median	187	213
Range	135–312	141–412
Presence of fistulae (no. of patients)	3	5
Extraintestinal manifestations (no. of patients)	12	10
Corticosteroid treatment (no. of patients)*	15	17
Aminosalicylate use*		
No. of patients	17	16
Dose (g/day)		
Median	3.0	3.0
Range	1.5–4.5	1.5–4.0
Age (yr)		
Median	26	31
Range	18–61	18–59
Sex (M/F)	14/3	11/6
Duration of disease (mo)		
Median	50	61
Range	6–212	6–240
Previous intestinal surgery (no. of patients)	9	5
Ferritin level (μg/liter)		
Median	107	129
Interquartile range	81–145	88–192
Total plasma protein level (g/liter)		
Median	71	73
Interquartile range	68–75	69–77
Albumin level (g/liter)		
Median	35	39
Interquartile range	29–41	31–44

*At the time of randomization.

Patients kept daily diaries in which they recorded the occurrence of specific symptoms used to calculate the Crohn's Disease Activity Index[12,13] or the Clinical Colitis Activity Index.[14] The Crohn's Disease Activity Index provides a calculated composite score that incorporates eight items: the number of liquid or very soft stools, the degree of general well-being, the degree of abdominal pain, the size of any abdominal mass, the hematocrit, body weight, extraintestinal manifestations, and the use of opiate-receptor–stimulating drugs for the control of diarrhea. Higher scores indicate more disease activity. Patients with a score below 150 are considered to be in clinical remission. Scores above 450 reflect severe active Crohn's disease. The Clinical Colitis Activity Index[14] incorporates seven clinical items: the number of stools, whether there is blood in the stools, an investigator's global assessment of the patient's symptomatic state, increased body temperature due to colitis, the degree of abdominal pain, the presence of extraintestinal manifestations, and the erythrocyte sedimentation rate. Patients with a Clinical Colitis Activity Index of 4 or less (on a scale of 0 to 16) are considered to be in remission.[14]

Serum erythropoietin levels were measured in frozen serum samples (−80°C) by a commercial radioimmunoassay (Bio-Merieux, Nörtingen, Germany). The lower limit of sensitivity was 4 U per milliliter. The amount of immunoreactive erythropoietin determined by the radioimmunoassay correlates with biologic activity as assessed by the polycythemic-mouse bioassay.[15,16]

To assess the immunologic activity of the disease, peripheral-blood mononuclear cells were isolated by density-gradient centrifugation over Ficoll–Hypaque (Pharmacia, Freiburg, Germany),[17] and monocytes were purified by substrate adherence.[7,18] Viability was more than 96 percent, and the cell suspension contained more than 97 percent monocytes. Monocytes were cultured at a concentration of 50,000 cells per milliliter in the presence of pokeweed mitogen (1 percent vol/vol),[7,18] and supernatants were collected after 24 hours. Concentrations of interleukin-1β were assessed with the use of a specific sandwich enzyme-linked immunosorbent assay (DPC/Biermann, Bad Nauheim, Germany).[7,18] All samples were analyzed in triplicate.

End Points and Measures of Efficacy

The primary measure of efficacy was an increase in hemoglobin levels by more than 1.0 g per deciliter (0.62 mmol per liter). End points were the completion of the 12-week treatment protocol and treatment failure — defined as a drop in the hemoglobin level of more than 2.0 g per deciliter (1.24 mmol per liter) to a value below 8.0 g per deciliter (4.96 mmol per liter), or any drop in hemoglobin to less than 6.5 g per deciliter (4.03 mmol per liter); the latter also indicated the need for transfusion. Criteria for withdrawal from the study were severe reactions at the injection site, major surgery, hemoglobin levels of 14.0 g per deciliter (8.69 mmol per liter) or higher, signs of hemolysis, intestinal blood loss leading to hospital admission, gastrointestinal ulcer disease, and withdrawal of consent. If 12 weeks of treatment were not completed, patients underwent the 12-week study-termination examination early.

Statistical Analysis

Normality was tested by the Shapiro–Wilk's W test.[19] The Student t-test was used to evaluate differences in hemoglobin values between the treatment groups, and Welch's unpaired t-test was used to compare serum erythropoietin concentrations. Differences in levels of cytokine secretion between the groups were tested with the Mann–Whitney U test. Treatment success (during the preliminary phase and erythropoietin treatment) was evaluated with the use of Kaplan–Meier statistics in life-table analyses, and differences were tested for statistical significance with the log-rank test.[20]

Results

Patient Group

Clinical and laboratory data on all outpatients with inflammatory bowel disease were reviewed. Of 334 patients with Crohn's disease, 88 were anemic (26 percent), with hemoglobin levels of ≤10.0 g per deciliter. Patients

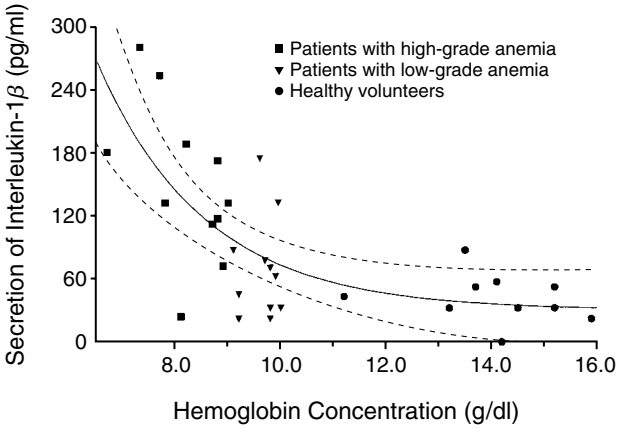

Figure 1. Relation of in Vitro Monocyte Secretion of Interleukin-1β to Hemoglobin Concentrations in 22 Patients with Inflammatory Bowel Disease and in Healthy Volunteers.

An exponential mathematical function describes the relation between hemoglobin levels in anemic patients with inflammatory bowel disease and monocyte secretion of interleukin-1β: $y = a_0 + a_1^{(-x/a_2)}$, with predicted 95 percent confidence intervals (dashed lines). Peripheral monocytes from 11 patients with high-grade anemia (hemoglobin levels below 9.0 g per deciliter) had significantly higher levels of interleukin-1β secretion (median, 132 pg per milliliter; interquartile range, 112 to 188) than those from 11 patients with low-grade anemia (hemoglobin levels of 9.0 to 10.0 g per deciliter; secretion of interleukin-1β, 62 pg per milliliter [32 to 87]; $P = 0.012$). Monocytes from 10 healthy volunteers had a median secretion of interleukin-1β of 37.5 pg per milliliter (interquartile range, 22 to 52), with a range of 0 to 87 pg per milliliter.

with anemia had greater clinical disease activity than those who were not anemic (Crohn's Disease Activity Index, 207±27 vs. 137±22; $P = 0.02$). Findings in 342 patients with ulcerative colitis were similar: 126 were anemic (37 percent) and had greater disease activity than the patients without anemia (mean [±SE] Clinical Colitis Activity Index, 8.7±2.1 vs. 3.7±1.8; $P = 0.04$). Ninety-one patients (34 with Crohn's disease and 57 with ulcerative colitis) entered the preliminary phase designed to identify patients with anemia refractory to sustained iron therapy. Of the remaining patients with anemia, 64 were not classified by their physicians as having anemia that was difficult to treat, 15 were not willing to participate, and 44 were excluded for other reasons (31 had used immunosuppressive drugs within the previous four months, 5 needed surgical treatment, 3 had suspected iron deficiency, 2 had impaired renal function, 2 lacked an approved method of contraception, and 1 had arterial hypertension).

Hemoglobin Levels and Immunologic-Disease Activity

In vitro monocyte secretion of interleukin-1β was assessed in 22 patients with anemia who had been randomly selected during the preliminary phase. Secretion was related to hemoglobin levels (Fig. 1). If patients were arbitrarily grouped according to the severity of their anemia, monocytes from 11 patients with severe anemia (hemoglobin level, ≤9.0 g per deciliter [5.59 mmol per liter]) had higher levels of in vitro secretion of interleukin-1β (median, 132 pg per milliliter; range, 24 to 280) than those from 11 patients with mild anemia (hemoglobin level, 9.0 to 10.0 g per deciliter) (median interleukin-1β concentration, 62 pg per milliliter; range, 22 to 174; $P = 0.012$) (Fig. 1). Monocytes from 10 healthy volunteers had a median secretion of interleukin-1β of 37.5 pg per milliliter (range, 0 to 87), which was lower than for the patients with anemia associated with inflammatory bowel disease ($P = 0.007$).

Serum Erythropoietin Levels in Patients with Inflammatory Bowel Disease and Anemia

Serum erythropoietin levels were assessed in 52 randomly selected outpatients with inflammatory bowel disease (34 had Crohn's disease and 18 had ulcerative colitis). The 18 patients with anemia associated with inflammatory bowel disease had higher serum erythropoietin levels (40.4±7.17 U per milliliter) than the 34 patients with inflammatory bowel disease without anemia (21.1±1.3 U per milliliter; $P = 0.002$) or 24 normal controls (13.7±1.2 U per milliliter; $P = 0.002$).

In models designed to test whether serum erythropoietin concentrations were adequate in relation to the degree of anemia, a linear regression was calculated between the logarithms of serum erythropoietin concentrations and the corresponding hematocrits.[21] The slope of the grade relates serum erythropoietin levels to the degree of anemia. We performed a linear regression analysis, including data on the 52 randomly selected patients with inflammatory bowel disease (with and without anemia) and, to permit definition of the normal response over a range of hematocrits, data on 24 healthy volunteers and 18 patients with anemia for reasons other than inflammation (e.g., blood loss following trauma or surgery) (Fig. 2). The slope for the control population (log erythropoietin = 3.446 − 0.0522 × hematocrit; $r = -0.88$; $P < 0.001$) was in the same range as has been described elsewhere.[21] The slope for patients with inflammatory bowel disease (log erythropoietin = 2.233 − 0.0255 × hematocrit; $r = -0.61$; $P = 0.007$) was lower

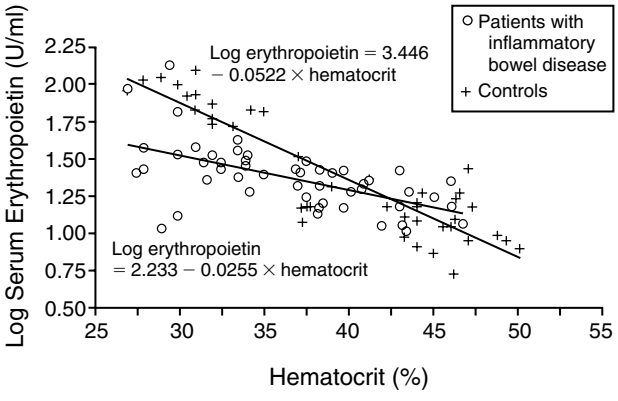

Figure 2. Relation of the Log Serum Erythropoietin Concentration to the Hematocrit.

In 52 patients with inflammatory bowel disease, the slope of the regression ($r = -0.61$, $P = 0.007$) was lower than in the controls ($r = -0.88$, $P < 0.001$), who included 24 normal volunteers and 18 patients with anemia for reasons other than inflammation (age- and sex-matched patients who were anemic because of traumatic blood loss) ($P = 0.03$).

than for the controls without inflammatory bowel disease (P = 0.03). Although anemia in inflammatory bowel disease is associated with increased erythropoietin levels, these levels were not as high as in patients with anemia of equal severity from noninflammatory conditions.

Erythropoietin Treatment of Patients with Anemia and Inflammatory Bowel Disease

Anemia refractory to the sustained iron treatment used during the preliminary selection phase of the study was more frequent (P = 0.007) in patients with Crohn's disease (19 of 34, 56 percent) than in those with ulcerative colitis (15 of 57, 26 percent). The base-line characteristics of the 34 patients who were randomized are shown in Table 1.

Hemoglobin levels in the 17 patients treated with erythropoietin increased from 8.81±0.27 g per deciliter (5.47±0.17 mmol per liter) to 10.52±0.41 g per deciliter (6.5±0.25 mmol per liter); hemoglobin concentrations in the 17 patients in the placebo group decreased from 8.69±0.11 g per deciliter (5.4±0.068 mmol per liter) to 7.84±0.33 g per deciliter (4.9±0.2 mmol per liter). Hemoglobin levels increased by 1.71±0.43 g per deciliter (1.06±0.27 mmol per liter) in the patients treated with erythropoietin, as compared with a decrease of 0.85±0.29 g per deciliter (0.53±0.18 mmol per liter) in the placebo group (P<0.001) (Table 2). A Kaplan–Meier analysis of treatment success is shown in Figure 3. Treatment with erythropoietin and iron was better than treatment with placebo and iron (rate of treatment success at day 84, 82 percent vs. 24 percent; P = 0.002). In the erythropoietin group, serum erythropoietin levels increased over the 12-week period by 22.4±8.1 U per milliliter, as compared with an increase of 7.1±7.4 U per milliliter in the placebo group (P = 0.17).

During the trial, there were five treatment failures in the placebo group and two in the erythropoietin group (P = 0.18).

Predictors of Response to Erythropoietin

Age, sex, corticosteroid use, clinical activity, site of disease, and serum concentrations of erythropoietin, ferritin, and C-reactive protein did not predict the response to erythropoietin (data not shown). We investigated whether the proinflammatory activity of monocytes from patients with inflammatory bowel disease predicted the response to erythropoietin in vivo. Monocyte secretion of interleukin-1β correlated inversely (r = −0.62) with changes in hemoglobin levels during 12 weeks of erythropoietin treatment (P = 0.07). Both of the patients in the erythropoietin group who did not respond to treatment had high proinflammatory activity (monocyte-supernatant interleukin-1β concentrations, 137 and 187 pg per milliliter; normal range, 0 to 87).

Table 2. Hemoglobin Levels.*

Variable	Erythropoietin Group (N = 17)	Placebo Group (N = 17)
Hemoglobin level (g/dl)		
Before treatment	8.81±0.27	8.69±0.11
After treatment	10.52±0.41	7.84±0.33
Difference in hemoglobin levels (g/dl)†	1.71±0.43	−0.85±0.29
Response after 6 weeks (% of patients)‡	65	12
Response after 12 weeks (% of patients)‡§	82	24

*Plus–minus values are means ±SE. To convert values for hemoglobin to millimoles per liter, multiply by 0.6206.

†P<0.001 for the comparison between the two groups.

‡A response was defined as an increase in hemoglobin of 1.0 g per deciliter or more.

§P = 0.002 for the comparison between the two groups.

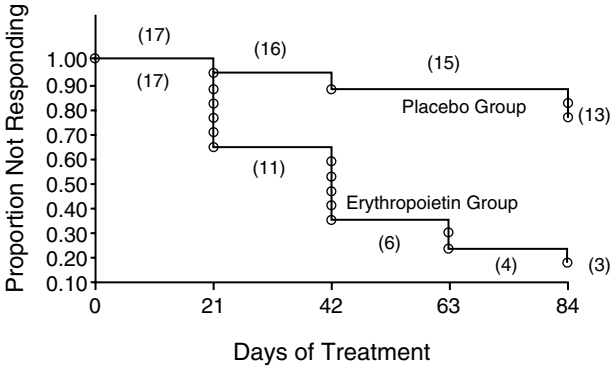

Figure 3. Kaplan–Meier Analysis of Treatment Success.

Treatment success was defined as an increase in hemoglobin of more than 1.0 g per deciliter. Eighty-two percent of patients receiving erythropoietin had responded by day 84, in comparison with 24 percent of patients in the placebo group. The numbers of patients remaining in each group are shown in parentheses. The log-rank test demonstrated the superiority of erythropoietin treatment as compared with placebo (P = 0.002).

Discussion

The development of anemia in patients with chronic disease caused by an impairment of iron use has been attributed in animal models[8-10] and in people[22] to a relative deficiency of erythropoietin. An enhanced production of proinflammatory mediators, including interleukin-1β, tumor necrosis factor α, and interferon γ, which may inhibit both the production of erythropoietin and the stimulatory effect of erythropoietin on the proliferation and maturation of erythroid precursors, may be an underlying pathophysiologic mechanism.[23]

We confirmed that serum erythropoietin levels in patients with a history of chronic anemia in inflammatory bowel disease, although higher than normal, are lower than in patients with anemia from noninflammatory disorders and are inadequate for the degree of anemia.[1,2] A relative deficiency of erythropoietin, despite normal renal function, may contribute to the development of chronic anemia in patients with inflammatory bowel disease.

We also demonstrated an association between the degree of anemia in inflammatory bowel disease and both clinical disease activity and in vitro proinflammatory-cytokine (interleukin-1β) production by monocytes, which is increased during acute intestinal inflammation.[4-7] We suggest the applicability of findings in animal models, which delineate clinical inflammation and proinflammatory-cytokine activity as key factors in the pathophysiol-

ogy of anemia in chronic diseases, to the pathophysiology of iron-resistant anemia in inflammatory bowel disease.

We studied whether substitution treatment with recombinant erythropoietin may be beneficial for patients with anemia associated with inflammatory bowel disease and refractory to iron therapy. We found that subcutaneous administration of pharmacologic doses[24] of erythropoietin twice a week, concomitantly with oral iron, increased hemoglobin concentrations in such patients, whereas there was a decrease in the placebo group, which received oral iron only.

Our analysis of the response to erythropoietin treatment and the proinflammatory activity of monocytes from patients with inflammatory bowel disease supports the initial hypothesis that proinflammatory cytokines may have a pivotal role in the development of anemia refractory to therapy in patients with inflammatory bowel disease: an inverse relation was seen between high proinflammatory activity (secretion of interleukin-1β by peripheral-blood monocytes) and the response to erythropoietin therapy. Moreover, both of the patients who failed to respond to erythropoietin were among those with high proinflammatory activity. It is possible that in some patients an impaired response to erythropoietin may be overcome by increasing the dosage.

Our results suggest that recombinant erythropoietin, in conjunction with oral iron, may be of use in patients with active inflammatory bowel disease and severe anemia refractory to iron therapy. It is important to determine whether the response to recombinant erythropoietin is dose-dependent. The possible role of proinflammatory mediators as modulators of the erythropoietin response should be characterized. Simultaneous assessment of total body iron and iron use should help determine the optimal dose and formulation of iron supplementation during erythropoietin treatment.

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