Impaired Erythropoietin Response to Anemia After Bone Marrow Transplantation

By Carole B. Miller, Richard J. Jones, Mariana L. Zahurak, Steven Piantadosi, William H. Burns, George W. Santos, and Jerry L. Spivak

Delayed erythroid recovery is common after bone marrow transplantation (BMT), with some patients continuing to require red blood cell (RBC) transfusion support for as long as 1 year. While the etiology is multifactorial, inadequate stimulation of erythroid progenitors by the erythroid growth factor, erythropoietin, may play a role. In this study, the erythropoietin response to anemia of 70 consecutive patients undergoing BMT at the Johns Hopkins Oncology Center was compared with the erythropoietin response in uncomplicated iron deficiency anemia. Erythropoietin levels were elevated for the degree of anemia early after BMT; however, at the time of marrow recovery, erythropoietin levels were significantly suppressed in both allogeneic and autologous BMT patients compared with the iron-deficient patients. Patients with acute graft-versus-host disease (GVHD) had a more marked suppression of the erythropoietin response to anemia. In the patients who remained anemic for extended periods of time (up to 12 months after BMT), an inadequate erythropoietin response to anemia persisted. Delayed erythroid recovery after BMT is associated with inadequate erythropoietin levels. Therefore, recombinant human erythropoietin may be useful in the treatment of the anemia associated with both autologous and allogeneic BMT.

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The presence of iron deficiency was established by either a serum ferritin level less than 30 μg/L in men or 10 μg/L in women, or by a serum iron level less than 65 μmol/L, a serum transferrin level greater than 4.10 g/L, and a transferrin saturation percentage of less than 20%. All controls had normal renal function.

Statistical evaluation. Erythropoietin levels were measured concurrently with hemoglobin levels in all patients. Because renal failure and renal insufficiency affect the erythropoietin response to anemia,28,29 for analysis of the effect of BMT on the erythropoietin response, patient samples were excluded when the serum creatinine was greater than 2.0 mg/dL. Analysis of covariance30 was used to model the association between erythropoietin and hemoglobin levels while controlling for the effects of the group (BMT v iron deficiency anemia, type of transplant, sex). Erythropoietin response was defined as the slope of erythropoietin level on hemoglobin. Pairwise differences between mean hemoglobin and serum immunoreactive erythropoietin levels were tested for statistical significance using the t-test. When the variances in comparison groups were not equal, corrections in the t statistic and degrees of freedom were made.31 Because erythropoietin levels were skewed, all analyses were performed after a logarithmic transformation. All P values reported are two-sided. Erythropoietin and hemoglobin levels for groups are reported as the mean ± SEM.

RESULTS

The patient characteristics are summarized in Table 1. Patients’ erythropoietin levels were appropriate for the hemoglobin levels pretransplant compared with iron-deficient controls (P = .5, data not shown). Erythropoietin levels were elevated in patients at 1 and 2 weeks after BMT, when compared with normal erythropoietin values for nonanemic patients and the normal erythropoietin response to anemia in the iron-deficient patients (Fig 1) (P = .002). The elevation in erythropoietin levels seen early after BMT occurred in both autologous and allogeneic BMT patients (P = .92) and was not influenced by preparative regimen (P = .5) or bilirubin level (P = .1).

At weeks 4 and 5 after BMT, erythropoietin levels were linearly correlated with the serum bilirubin (P = .01, r = .62); as seen in chronic renal failure, patients with hepatic dysfunction had an inappropriately high erythropoietin response. When patient samples with concurrent bilirubin level greater than 2.0 mg/dL were excluded, the erythropoietin response to anemia was suppressed in patients after both allogeneic BMT at week 4 (P = .04) and week 5 (P = .003) and autologous BMT at week 4 (P = .01) and week 5 (P = .002) compared with iron-deficient patients (Fig 2). Also, the erythropoietin to hemoglobin ratios at weeks 4 and 5 were significantly decreased compared with the pretransplant ratios for autologous (P = .005) and autologous (P = .03) BMT patients.

Although there was no difference in the erythropoietin response to anemia between the allogeneic and autologous BMT patients at weeks 4 and 5 after BMT (P = .9), the presence of acute GVHD in the allogeneic BMT patients was associated with a more severe depression in the erythropoietin response to anemia (P = .015) (Fig 2). Cytomegalovirus status or the use of amphotericin B did not affect the erythropoietin response to anemia (P = .6, P = .3, respectively). As all allogeneic BMT patients received cyclosporine A for at least 6 months, the effect of cyclosporine A on the erythropoietin response to anemia could not be determined in this group. However, one third of the autologous BMT patients received cyclosporine A for

Table 1. Patient Characteristics

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Abbreviations: AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin's leukemia; HD, Hodgkin's disease; Prep, preparative regimen; BU, busulfan; CY, cyclophosphamide; TBI, total body irradiation; VP16, etoposide; CMV, cytomegalovirus.
28 days as part of a phase II trial of the induction of autologous GVHD.32 Cyclosporine A did not appear to affect the erythropoietin response to anemia ($P = .9$) in the patients receiving autologous transplants.

Of the 70 patients, 46 (29 allogeneic, 17 autologous) returned for a 6-month follow-up visit. The reason for not returning was transplant-related death (11 patients), relapse of disease (9 patients), or failure to return at the specified time (4 patients). Twenty-eight patients who returned for the 6-month visit remained anemic (hemoglobin, <11 g/dL). Allogeneic BMT patients were more likely to be anemic than autologous BMT patients (22 of 29 v 6 of 16, $P = .007$, $\chi^2$ test) and the mean hemoglobin was lower in the allogeneic BMT patients than in the autologous BMT patients ($10.3 \pm 1.2$ v $11.1 \pm 1.3$, $P = .05$, $t$-test). This could not be explained by poorer renal function in the allogeneic patients, as there was no difference in mean serum creatinine ($1.4 \pm 0.8$ in the allogeneic patients v $1.2 \pm 0.3$, $P = .18$). There was also no correlation between hemoglobin and serum creatinine in this group ($P = .56$). Within the allogeneic BMT patients, chronic GVHD did not influence the mean hemoglobin ($10.2 \pm 1.2$ v $10.4 \pm 1.4$, $P = .5$, $t$-test) or occurrence of anemia ($P = .58$).

The erythropoietin response in the anemic BMT patients remained severely depressed compared with iron-deficient patients ($P = .002$) (Fig 3). The erythropoietin response was not influenced by the type of graft ($P = .15$) or the presence of chronic GVHD ($P = .3$).

Thirty-five patients returned for a follow-up visit at 12 months. The reason for not returning for evaluation was relapse in four patients, transplant-related death in two patients, and lack of a timely 12-month follow-up visit in five patients. While none of the seven autologous patients were anemic, 10 of the 28 allogeneic patients who returned for the 12-month visit were anemic ($P = .1$). The erythropoietin response in the anemic patients remained depressed compared with iron-deficient patients ($P = .05$) (Fig 3).

**DISCUSSION**

Patients undergoing BMT display a characteristic erythropoietin response. Changes in mean erythropoietin and hemoglobin levels over time in the absence of liver dysfunction are summarized in Fig 4. Most patients have normal erythropoietin responses before BMT. We found that erythropoietin levels were elevated weeks 1 and 2 after

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**Fig 2.** Erythropoietin/hemoglobin relationship weeks 4 and 5 after BMT (allogeneic BMT without GVHD, $\triangle$; allogeneic BMT with GVHD, $\square$; autologous BMT, $\bigcirc$) compared with erythropoietin/hemoglobin relationship in iron-deficient patients represented by the shaded area.

**Fig 3.** Erythropoietin/hemoglobin relationship in anemic patients at 6-month ($\bullet$) and 12-month ($\blacklozenge$) follow-up visits compared with erythropoietin/hemoglobin relationship in iron-deficient patients represented by the shaded area.

**Fig 4.** Summary of mean ± SD erythropoietin (——) and hemoglobin (—) levels in the BMT patients over time. *Erythropoietin to hemoglobin ratios that were significantly different from pretransplant ratios.
BMT without significant anemia. The levels decrease over the next weeks and, at the time of projected marrow recovery (weeks 4 and 5 after transplant), erythropoietin levels were depressed compared with iron-deficient anemic patients and the hemoglobin-erythropoietin relationship seen pretransplant. The inadequate erythropoietin response to anemia persists up to 1 year in some patients and is thus likely to be at least partially responsible for the prolonged anemia after BMT.

The etiology of the inadequate erythropoietin response after BMT (week 4 up to 1 year posttransplant) is unclear, but probably multifactorial. The only factor evaluated that appeared to blunt the erythropoietin response to anemia was acute GVHD. This may be related to the cytokines released as a result of inflammation associated with GVHD or to the treatment of GVHD. While patients with acute GVHD displayed a depressed erythropoietin response to anemia after allogeneic BMT, the erythropoietin response to anemia was also blunted, although to a lesser degree, in allogeneic BMT patients without clinically evident acute GVHD. Chronic GVHD did not affect either the occurrence of anemia or the erythropoietin response to anemia. Tumor necrosis factor, which has been shown to be elevated after BMT, especially in relation to GVHD, can suppress erythropoietin production in an in vitro model system.

Alkylating agents, including cyclophosphamide, have previously been shown to decrease the ability of the kidney in dogs to make erythropoietin in response to a cobalt challenge, and all patients in this study received one or more alkylating agents. While we controlled for clinically significant renal dysfunction, subclinical changes in renal function may be important in the erythropoietin response to anemia. Nephrotoxic drugs such as amphotericin B or cyclosporine A have been suggested to be important in the suppression of the erythropoietin response to anemia; however, we could not distinguish an association between either amphotericin B or cyclosporine A use and the erythropoietin defect in these patients at weeks 4 and 5 after BMT. Showing an effect of these drugs in this patient population may be difficult, because only one of the 70 patients did not receive at least one of these drugs during the immediate posttransplant period.

Contrary to reports from other centers, we found that the erythropoietin response to anemia was suppressed in autologous BMT patients, as well as in allogeneic BMT patients. Our autologous BMT population was different in that 30% of the autologous BMT patients received cyclosporine A for 28 days in an attempt to induce autologous GVHD. Although we were unable to show that treatment with cyclosporine A after autologous BMT was a predictor of an inadequate erythropoietin response to anemia, there are animal data to suggest that cyclosporine A can inhibit the erythropoietin response to anemia even in the absence of nephrotoxicity. We have found that allogeneic BMT patients were more likely to by anemic at the 6-month and 12-month visits than autologous BMT patients. Although we could not determine a reason for this difference, it could be due to the longer treatment with cyclosporine A in the allogeneic population.

The elevated erythropoietin levels without anemia early after BMT have been described by others and suggest an alternative method for erythropoietin stimulation in these patients. We did not find that the increased erythropoietin levels were associated with preparative regimen, type of transplant, or clinical hepatic injury measured by serum bilirubin. As there is no stored reserve of erythropoietin in humans, the increased levels must reflect either increased production, decreased elimination, or decreased utilization. Decreased utilization of erythropoietin by erythroid progenitors that are absent during marrow aplasia has been suggested as an etiology for these high erythropoietin levels. However, it is unlikely that decreased target cells are the etiology for this markedly increased level in the absence of increased production or decreased elimination, as the high erythropoietin levels fall off rapidly during the first 2 weeks after transplant despite marrow aplasia that may persist for 4 weeks or longer.

Hepatic injury after BMT can cause marked increases in serum erythropoietin, as also described in patients with chronic renal failure. In this study, erythropoietin levels at weeks 4 and 5 after BMT were correlated only with bilirubin elevations, instead of the normal relationship between hemoglobin and erythropoietin levels. The liver is a source of approximately 10% of nonfetal erythropoietin production, and it is unclear whether the source of the high erythropoietin levels in this patient population is from the liver or kidney.

These data of the erythropoietin response to anemia in a large patient population confirm previous observations that patients after allogeneic BMT have an inadequate stimulus for erythroid progenitor proliferation at the time of marrow recovery and presents data to support a defect in autologous BMT patients as well. This suggests that exogenous erythropoietin may decrease the transfusion requirement after BMT as it has in other anemias (chronic renal failure, human immunodeficiency virus infection, cancer, and chemotherapy) where an inappropriately low erythropoietin response to anemia is found. Studies are currently underway to evaluate the response of the anemia after BMT to treatment with recombinant human erythropoietin.

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