Recombinant Human Growth Hormone Promotes Hematopoietic Reconstitution after Syngeneic Bone Marrow Transplantation in Mice

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ABSTRACT

Recombinant human growth hormone (rhGH) was administered to mice after syngeneic bone marrow transplantation (BMT) to determine its effect on hematopoietic reconstitution. BALB/c mice were given 10 μg intraperitoneal injections of rhGH every other day for a total of 10 injections following syngeneic BMT. Mice that received rhGH exhibited significant increases in total hematopoietic progenitor cell content (colony-forming unit-culture) in both bone marrow and spleen. Erythroid cell progenitor content (burst-forming unit-erythroid) was also significantly increased after rhGH treatment. Analysis of peripheral blood indicated that administration of rhGH resulted in significant increases in the rate of white blood cell and platelet recovery. Granulocyte marker 8C5+ cells were also increased in the bone marrow and spleens of treated mice. Red blood cell, hematocrit, and hemoglobin levels were increased at all time points after rhGH treatment. No significant pathologic effects or weight gain were observed in mice receiving repeated injections of 10 μg rhGH. Thus, rhGH administration after syngeneic BMT promoted multilineage hematopoietic reconstitution and may be of clinical use for accelerating hematopoiesis after autologous BMT. Stem Cells 1998;16:193-199

INTRODUCTION

Autologous bone marrow transplantation (BMT) is currently used in the treatment of a variety of neoplastic diseases. However, there are several problems associated with autologous BMT. The period of bone marrow aplasia leaves patients at risk for opportunistic infections, and the immunosuppression associated with the cytotoxic therapy results in a greater risk of tumor recurrence [1]. Growth hormone (GH) exerts a variety of growth-promoting effects on the body. Primarily associated with its anabolic effects, GH has also been implicated in immune development and function [2]. GH has been previously demonstrated to promote both erythropoiesis and granulopoiesis in vitro [3, 4], although little is known in regard to its effects in vivo. Recently, we reported that treatment of mice with recombinant human growth hormone (rhGH) could partially reverse the myelosuppressive effects of azidothymidine (AZT) [5]. Treatment with rhGH of DW/J dwarf mice, which are deficient in GH, likewise resulted in an improvement in their hematological status [6]. We therefore wanted to assess the effects of GH treatment on hematopoietic recovery following syngeneic BMT (SBMT) in mice. We report here that rhGH promotes hematopoietic reconstitution in mice when administered after SBMT, affecting multiple hematopoietic lineages at various stages in differentiation. Therefore, rhGH may be of potential clinical use after high-dose chemotherapy and autologous BMT to promote hematopoietic recovery.

MATERIALS AND METHODS

Mice

BALB/c mice were obtained from the Animal Production Area (NCI-FCRDC; Frederick, MD). Mice were not used until 8-12 weeks of age.

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Irradiation and SBMT

Recipient BALB/c mice received 850 cGy total body irradiation by exposure to a $^{137}$Cs source and were transplanted with $1 \times 10^6$ normal BALB/c bone marrow cells (BMC) i.v. There were three to five mice per group, and each experiment was performed three to four times.

Treatment with Hormones

Irradiated and transplanted mice received either 10 µg rhGH or Hanks’ balanced salt solution (HBSS) (control) at day 1 after SBMT. rhGH, provided by Genentech (San Francisco, CA.), was resuspended in 0.2 ml HBSS and injected intraperitoneally (i.p.) every other day until the mice were assayed or had received a total of 10 injections over 20 days. Mice were weighed weekly. In some experiments, 10 µg of ovine GH (ovGH) was injected using the same schedule as rhGH. Purified ovGH was provided by the repository supported by the National Institute of Diabetes and Digestive and Kidney Diseases, the Center for Population Research of the National Institute of Child Health and Human Development, and the Agriculture Research Service of the U.S. Department of Agriculture, as well as the University of Maryland School of Medicine, Baltimore, MD.

Hematopoietic Analysis

Blood was collected from mice via the lateral tail vein, using EDTA as an anticoagulant. CBC analysis of the peripheral blood samples was performed using an HC 820 Hematology Analyzer (Danam Electronics, Inc.; Dallas, TX). MetPath (Rockville, MD) performed differential blood cell analysis. The cellularities of bone marrow, spleen, and thymus were assayed using a Coulter Counter (Coulter Electronics; Hialeah, FL).

Assay for In Vitro Hematopoiesis

Spleen cells and BMC obtained from one tibia were washed and resuspended in Iscove’s modified Dulbecco’s medium supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and antibiotics. For the colony-forming unit-culture (CFU-C) assay [5], cells were plated in 0.3% bactoagar (Difco Laboratories; Detroit, MI) in 35-mm Lux petri dishes (Miles Laboratories, Inc.; Naperville, IL) at a concentration of $1 \times 10^6$ spleen cells or $2 \times 10^5$ BMC per plate. Colony formation was stimulated with predetermined optimal doses of 10 ng/ml recombinant murine granulocyte macrophage-colony-stimulating factor (rmGM-CSF) (Amgen Corporation; Thousand Oaks, CA) or 10 ng/ml recombinant murine interleukin-3 (rmIL-3) supplied by the Biological Response Modifiers Program Repository (Frederick, MD). Plates were incubated at 37°C for 7 days in 100% humidity, with a 5% CO$_2$ atmosphere, and then colonies were counted. A colony was defined as a clustered growth of more than 50 cells. For the burst-forming unit-erythroid (BFU-E) assay the suspension included: 1.5 ml cells, 1.5 ml FBS, 0.5 ml 10% BSA, 0.5 ml of 1.0 mM 2-mercaptoethanol, 0.5 ml erythropoietin (Epo), and 0.5 ml rmIL-3 [7]. The final concentrations were 2 U/ml Epo, 20 ng/ml rmIL-3, and $1 \times 10^5$/ml cells. BFU-E colonies were scored after 12 days of incubation. A BFU-E colony was defined as a group containing 50 or more benzidine-positive cells.

Flow Cytometry Analysis (FCM)

Spleenic, thymic, and bone marrow single-cell suspensions were prepared, stained, and analyzed by FCM analysis as previously described [6]. The fluorescence isothiocyanate-labeled anti-8C5 (granulocyte marker) antibody was obtained from Becton-Dickinson (Mountain View, CA). Each fluorescence study had directly labeled negative isotype controls of normal rat immunoglobulin.

Statistical Analysis

A Student’s t-test was performed to determine if values differed significantly ($p < 0.05$ or $p < 0.01$).

RESULTS

Hematopoietic Progenitor Cell Content in Mice Following rhGH Treatment after SBMT

To determine whether rhGH affects hematopoietic engraftment and reconstitution following SBMT, BALB/c mice were injected with different doses of rhGH every other day beginning the first day after lethal irradiation and BMC transfer. The progenitor cell content as determined by CFU-C significantly increased in mice that received rhGH compared with the HBSS-injected controls (Fig. 1). Because mice that received 10 µg of rhGH had greater hematopoietic progenitor cell content than those that were injected with 1 µg but were not significantly different from the animals injected with 100 µg, we used the 10 µg dose in the later experiments. No toxicity was observed at any amount of GH administered. Because hGH also binds murine PRL receptors [8], we determined whether these effects were specific to binding of GH receptors by injecting mice following SBMT with ovGH, which does not bind murine PRL receptors [8] (Fig. 2). Treatment of mice with ovGH exerted similar effects on CFU-C content as rhGH, indicating that rhGH exerts its hematopoietic effects, at least in part, through binding of GH receptors in vivo.
Although the effects of ovGH were somewhat lower than those observed with rhGH, the differences were not significant. The data, however, do not exclude the possibility that rhGH stimulation of PRL receptors contributes to the observed effects. Because GH has been previously demonstrated to affect erythroid lineage cells in vitro [3], we examined the effects of rhGH on BFU-E progenitor formation after SBMT. The BFU-E assay of spleen and BMC demonstrated that beginning at day 10, the mice that were treated with rhGH had significantly greater numbers of erythroid progenitor cells than the controls (Fig. 3). The total BMC BFU-E progenitors were increased 2.5-fold at day 10 ($p < 0.01$) and 2.2-fold at day 15 ($p < 0.01$). The total splenic BFU-E progenitors were increased 2.9-fold at day 10 ($p < 0.01$) and 1.3-fold at day 15 ($p < 0.05$). The results thus indicated that treatment with rhGH promoted BMC engraftment, resulting in improved development of hematopoietic progenitor cells.

**Peripheral White Blood Cell (WBC) Counts**

Because GH has been shown to stimulate granulopoiesis in vitro [4], we examined whether treatment of mice with rhGH after SBMT would result in an improvement in the peripheral WBC counts. CBC analysis indicated that administration of 10 µg rhGH after SBMT resulted in significant increases in WBC counts (Fig. 4). Through FCM analysis, we found that 8C5+ cell (granulocyte lineage) content in both bone marrow and spleen were augmented by rhGH administration (Fig. 5). The total BMC 8C5+ cell content increased 2.6-fold at day 14 ($p < 0.01$) and 1.7-fold at day 21 ($p < 0.01$), and the total splenic 8C5+ cells increased 2.1-fold at day 14 ($p < 0.01$) and 1.4-fold at day 21 ($p < 0.01$). The differential analysis of peripheral blood also showed that the percentage (Fig. 6A) and absolute number (Fig. 6B) of granulocytes were increased in rhGH-treated mice. Whereas the percentage of lymphocytes was decreased ($p < 0.05$) in the rhGH-treated animals, compared to control mice (Fig. 6A), the total number of lymphocytes was slightly increased following hormone treatment (Fig. 6B). This indicates that rhGH treatment can improve granulocyte recovery after SBMT.

**Red Blood Cell (RBC) Counts**

Because we detected increases in BFU-E after rhGH treatment, we examined...
whether administration of rhGH also improved the recovery of RBC after SBMT. Treatment with 10 µg of rhGH every other day significantly augmented the RBC recovery from day 15 to day 21 following SBMT (Fig. 7A), hemoglobin (HGB) concentration from day 8 to day 18 (Fig. 7B), and hematocrit (HCT) from day 15 to day 21 (Fig. 7C), demonstrating that rhGH accelerates the recovery of mature erythroid cells in the peripheral blood.

Platelet (PLT) Recovery

Thrombocytopenia remains a significant cause of morbidity, expense, and prolongation of hospitalization in patients receiving high-dose chemotherapy. We therefore examined the effects of rhGH on the recovery of PLT in the peripheral blood. As shown in Figure 8, the peripheral blood PLT counts of rhGH-treated mice were significantly increased at days 15 to 28 compared with the controls. At day 14 after SBMT, there was a dose-dependent increase in platelet count (data not shown), although the mice that received 100 µg rhGH showed no significant difference from those that received the 10 µg. Additionally, no significant increase in body weight was noted as a consequence of the 10 µg rhGH treatment regimen after SBMT (data not shown), indicating that rhGH exerted its hematopoietic...
growth-promoting effects in vivo at doses that did not promote body growth.

**DISCUSSION**

GH exerts a variety of biological effects in vivo and has been suggested to affect hematopoietic cells [2-6]. The data presented here indicate that administration of rhGH to mice following SBMT promoted hematopoietic reconstitution, as determined by hematopoietic progenitor cell contents of bone marrow and spleen, and WBC, PLT, RBC, and HCT recoveries in the peripheral blood.

We have previously determined that DW/J dwarf (GH-deficient) mice exhibited decreased hematologic parameters [6]. Subsequent treatment of these mice with rhGH improved their hematological status as indicated by increased HCT, PLT, and WBC levels [6]. Additionally, the anemia and neutropenia resulting from AZT treatment in mice were improved by administration of rhGH [5]. The data reported here support and extend these findings, as significant increases in WBC, PLT, RBC, and HCT recoveries were obtained in mice receiving rhGH after SBMT. It is also important to note that the hematopoietic growth-promoting effects of rhGH after SBMT occurred at a dose and schedule that did not result in toxicity or weight gain. It was recently reported that systemic administration of IL-11 after SBMT [9], or IL-6 following radiotherapy [10, 11] also increases PLT recovery. In addition, treatment with IL-1α improved PLT recovery after high-dose carboplatin therapy [12]. In a clinical setting, systemic administration of these cytokines may produce toxic side effects [13]. The hematopoietic...
effects of GH may not be as pronounced in vivo as other hematopoietic growth factors, but multiple lineages appear to be affected by GH. Additionally, the advantage of rhGH relative to other cytokines is its reduced systemic toxicity. Clinical GH administration has been given to induce weight gain and, when administered to HIV-infected patients, has been shown to be well tolerated with moderate headache, fatigue, myalgia, arthralgia, and edema being observed in some patients [14]. It will be of interest to assess the effects of GH in combination with other hematopoietic growth factors such as G-CSF or GM-CSF to determine if potential synergistic effects could be achieved.

The mechanism by which rhGH promotes hematopoietic reconstitution after SBMT is not yet clear. The stimulatory effects of this hormone on hematopoietic progenitor cells may be mediated directly by induction of progenitor cell proliferation [3, 4], or indirectly by improvement of the bone marrow cell response to growth-promoting factors. We are currently examining the effects of GH on cytokine production by stromal cell lines. Additionally, we are using lineage-negative purified hematopoietic progenitor cells to determine whether GH directly promotes hematopoietic growth in vitro. Due to the pleiotropic effects of GH, it is likely that the effects on hematopoiesis that we detected are due to a combination of these mechanisms. Additionally, many of the growth-promoting effects of rhGH are mediated by insulin-like growth factor 1 (IGF-1) [15-17]. Recent studies have determined that IGF-1 also promotes hematopoiesis after in vivo administration [18]. Therefore, the hematopoietic effects of rhGH administration in vivo may be partly due to the induction of IGF-1 release. Human GH also binds the murine PRL receptor [8], and some of the in vivo effects of rhGH may be mediated through this receptor. We have preliminary results indicating that PRL also exerts significant hematopoietic growth-promoting properties when administered to mice after SBMT (manuscript in preparation). However, the experiments done with ovGH (Fig. 2), which does not bind the murine PRL receptor [8], indicated that at least some of the hematopoietic effects of rhGH are specific to GH receptor-binding activity. It may be difficult to ascertain all the potential mechanisms by which GH exerts its hematopoietic growth-promoting effects in vivo.

Because GH has significant effects on the immune system [2], more work needs to be performed to assess the long-term effects of rhGH on immune reconstitution after SBMT. We have previously found that treatment of human/mouse chimeras with rhGH resulted in a high incidence of Epstein-Barr virus-induced human B-cell lymphomas in mice with severe combined immune deficiency [19]. Recurrence of the underlying malignancy remains a major cause of failure of autologous BMT used for the treatment of cancer [1]. Therefore, caution must be taken when using rhGH to enhance hematopoietic recovery when autologous BMT is used for cancer treatment because rhGH or IGF-1 may promote tumor growth. However, the responses of various lineages and developmental stages of hematopoietic cells to systemic administration of rhGH suggest that this hormone may be of potential therapeutic use in augmenting hematopoietic recovery after myelosuppressive therapy.

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Animal care was provided in accordance with the procedures outlined in “The Guide for the Care and Use of Laboratory Animals.”
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