

Recombinant Human Granulocyte–Macrophage Colony-stimulating Factor (GM-CSF) Shortens the Period of Neutropenia after Autologous Bone Marrow Transplantation in a Primate Model

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Abstract

The effect of granulocyte–macrophage colony-stimulating factor (GM-CSF) on hematopoietic reconstitution after autologous bone marrow transplantation was evaluated in a primate model. Animals were given a continuous intravenous infusion of recombinant human GM-CSF for several days both before and after transplantation or only after the transplant procedure. Marrow ablation was accomplished by total body irradiation. In both groups of animals, the neutrophil count reached 1,000/mm³ by 8–9 d posttransplant compared with an interval of 17 and 24 d for two concurrent controls. After withdrawal of GM-CSF, neutrophil counts fell to values comparable to those observed in untreated controls. Accelerated recovery of platelet production was also observed in four of the five animals. Two additional animals were initially given GM-CSF several weeks posttransplantation because of inadequate engraftment. Prompt and sustained increases in neutrophil and platelet counts were observed. We conclude that GM-CSF may be useful in accelerating bone marrow reconstitution.

Introduction

Neutropenia is accompanied by an increased risk for bacterial and fungal infection. Decreased neutrophil production may reflect intrinsic bone marrow disease, e.g., aplastic anemia or leukemia, or occur as a result of therapeutic interventions such as bone marrow transplantation or chemotherapy. The incidence and severity of infection is directly related to the duration and severity of neutropenia (1). A safe and effective means to accelerate bone marrow regeneration is likely to be of major clinical benefit.

Several hematopoietic growth factors, products of T lymphocytes, monocytes, and marrow stromal cells, appear to interact to modulate production of mature blood cells (2). Four such factors, interleukin 3 (IL-3)¹ (3), granulocyte–monocyte

colony-stimulating factor (GM-CSF) (4), granulocyte colony-stimulating factor (G-CSF) (5, 6), and macrophage–colony-stimulating factor (M-CSF) (7) have been defined by biochemical and molecular cloning techniques. In general, the hematopoietic factors are not lineage specific (2, 8–12). These factors are required for proliferation and differentiation of progenitor cells into mature blood cells in vitro and presumably in vivo, and have pleotropic effects on the function of mature cells (2, 13–15). Hematopoietic growth factors are thought to be produced and act locally within the marrow microenvironment in supporting hematopoiesis (16). Both GM-CSF and another multipotential factor, IL-3, have been shown to have significant effects on hematopoiesis when administered parenterally to animals (17–19). A striking increase in neutrophil number accompanied by increases in the number of monocytes, eosinophils, lymphocytes, and reticulocytes was observed when GM-CSF was given to monkeys (17). These observations provided the stimulus to define the effect of GM-CSF administration during bone marrow regeneration after autologous bone marrow transplantation.

Methods

Primate model. The experimental protocol was approved by the NHLBI Animal Study Committee and the animals were maintained according to the guidelines of the Committee on Care and Use of Laboratory Animals, National Research Council. Juvenile Rhesus monkeys (*Macaca mulatta*) weighing 3.5–5.0 kg were jacketed and maintained on a tether throughout the study period. A Broviac catheter was inserted via a jugular vein with the tip of the catheter lying in the superior vena cava or right atrium; the exit site was in the middle of the back. The animal subsequently was maintained on a continuous intravenous drip. Each animal was anesthetized daily with intravenous ketamine hydrochloride. Weight and temperature were determined, a blood sample was obtained via the catheter, and the intravenous tubing and solutions were changed. Standard red cell cross-matching techniques (20) were used to identify 10 donors for each recipient. Red cells and platelets were administered as needed. Hyperalimentation, potassium chloride, and antibiotics were given according to standard clinical indications. Animal survival till 60 d was routinely achieved but radiation pneumonitis and/or persistent gastrointestinal dysfunction limited long-term survival.

Autologous bone marrow transplantation. Bone marrow cells were aspirated by multiple punctures around each ischial tuberosity and from both femurs via a single puncture through the flexed knee joint. Mononuclear cells recovered by density centrifugation, were washed twice and resuspended in 50 ml of Dulbecco's phosphate-buffered saline containing 20% autologous plasma. Immediately after bone marrow aspiration, the animals received 1,200 rad of absorbed total body irradiation (12 rad/min). Within 2 h, each animal received an intravenous infusion of au-

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1. Abbreviations used in this paper: GM-CSF, granulocyte–monocyte colony-stimulating factor; IL-3, interleukin 3.

Table I. Summary of Study Parameters

	Animal No.	Pretransplant			Transplant		Posttransplant	
		GM-CSF infusion	Blood counts		Mononuclear	GM-CSF infusion	Neutrophils	Platelets
		days*	per mm ^{3†}	per mm ^{3†}	cells/kg	d [‡]	>1000/mm ³	>50,000/mm ³
			Neutrophils	Platelets				
I. Pre- and posttreatment	110	19	46,300	438,000	5.0 × 10 ⁸	11	Day 9	Day 11
	265	10	39,100	321,000	2.3 × 10 ⁸	9	9	14
	274	13	29,000	655,000	2.2 × 10 ⁸	12	8	16
II. Posttreatment	8107	—	3,500	331,000	3.6 × 10 ⁸	17	9	23
	7715	—	3,570	398,000	2.0 × 10 ⁸	10	9	14
III. Controls	0092	—	3,600	216,000	2.4 × 10 ⁸	—	17	21
	829	—	1,950	414,000	2.3 × 10 ⁸	—	24	22

* The GM-CSF infusion was discontinued on the day the transplant was performed (day 0). † Average of values obtained during 7 d before transplantation. ‡ Treatment with GM-CSF was initiated on day 2 posttransplantation except for animal 110 on whom the infusion was started on day 3.

tologous bone marrow cells to initiate reconstitution (Table I). Complete blood counts, reticulocyte counts, and serum chemistries were monitored daily using standard techniques.

GM-CSF administration. Recombinant human GM-CSF, synthesized by Chinese hamster ovary cells, was purified as previously described and shown to be free of endotoxin (4). A stock solution with a specific activity of $1\text{--}2 \times 10^6$ U/mg was diluted and given by continuous intravenous infusion at the rate of 50 U/min per kg (17). A loading dose equivalent to 20% of the daily dose was given initially.

Results

Effect of GM-CSF on neutrophil numbers. All five animals that received GM-CSF after infusion of autologous bone marrow cells exhibited accelerated return of neutrophil production (Figs. 1 and 2). By day 8 or 9 each animal had a neutrophil count of $> 1,000/\text{mm}^3$, whereas the two control animals did not reach this level until day 17 or 24 (Table I). Each treated animal exhibited a fall in neutrophil count immediately after the GM-CSF infusion was discontinued (Figs. 1 and 2). In general the counts fell to levels expected for that time in the recovery phase, providing direct evidence of a GM-CSF effect, with a subsequent increase as recovery progressed. One animal (274, Fig. 2B) had a fall in neutrophil count during the GM-CSF infusion (day 10). Because the animal was febrile, sepsis was suspected and antibiotics were initiated. The neutrophil count increased again (days 13–15) while the GM-CSF infusion was continued. The first three animals studied received GM-CSF for 10, 13, or 19 d before the transplant with a 10–15-fold increase in neutrophil numbers (Table I). There was no apparent effect on the time of onset of significant neutropenia or the rate of recovery of neutrophil numbers compared to the other two treated animals (Figs. 1 and 2, Table I).

Effect of GM-CSF on other hematological parameters. There appeared to be an acceleration of platelet recovery in four of the five animals that received GM-CSF compared with the two control animals (Table I). This effect was most striking in animals 110 (Fig. 1) and 274. Reticulocyte recovery to $> 40,000/\text{mm}^3$ was no different between the controls and treated animals. During GM-CSF administration, differentials revealed 80–95% neu-

trophils. Striking increases in monocyte or eosinophil numbers were not observed.

GM-CSF improves hematopoiesis in poorly reconstituted transplant recipients. Striking increases in neutrophil and platelet counts were observed during and following a brief course of GM-CSF in two animals that were pancytopenic 27 or 46 d after autologous bone marrow transplantation (Fig. 3). Bone marrow biopsies from the posterior iliac crest were hypocellular before

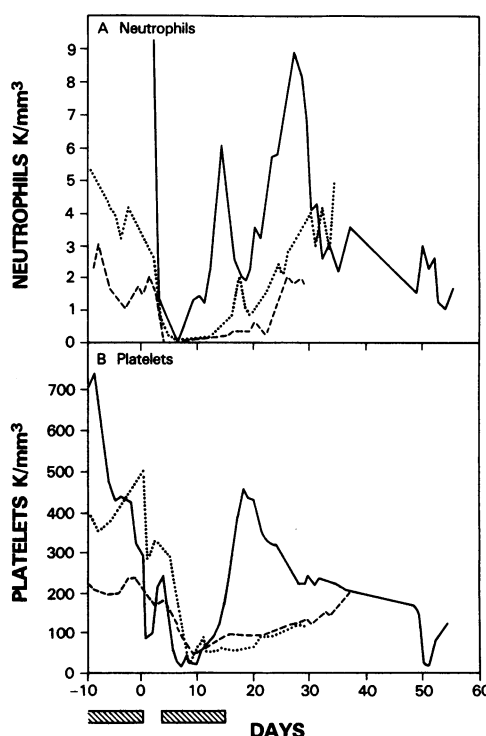


Figure 1. Reconstitution of neutrophil and platelet production in the initial animal (110) treated with GM-CSF. The period of GM-CSF administration is indicated below; pretreatment was started 19 d before the bone marrow transplantation on day 0. Comparison is made to counts in the two control animals 0092 and 829 (dotted and dashed lines, respectively).

factor administration. Cellularity was increased in a second specimen obtained 7 d after GM-CSF was started. These animals were part of a study in which transfer of the adenosine deaminase gene into stem cells had been attempted with retroviral vectors (21).

Untoward effects of GM-CSF. All five animals achieved reconstitution of each lineage with adequate blood counts during the 60-d period of observation. Thrombocytopenia in animal 110 (Fig. 1) at days 51–52 occurred when adequate megakaryocytes were present in the marrow suggesting transient accelerated platelet destruction. Two of the three animals treated with GM-CSF before and after marrow transplantation exhibited diffuse edema. Diarrhea, hypoalbuminemia, and hypokalemia, usually present to variable degrees in the posttransplant period, were more severe in the animals that developed edema. Recovery occurred after GM-CSF was discontinued and intravenous alimentation and potassium replacement were given.

Discussion

Our animal studies suggest that GM-CSF can be given safely with a significant increase in neutrophil number during bone

marrow regeneration without compromise of marrow engraftment or major untoward effects. Human trials will be required to define optimal dose and schedule of administration and to evaluate clinical benefit with respect to prevention of infection. These studies were designed to explore the effect of the factor on peripheral blood counts and do not address the issue of clinical efficacy. Since leukemic cells are known to respond to GM-CSF (2), the factor should not be used in such patients.

GM-CSF is a multilineage hemopoietin with documented ability to stimulate formation of colonies by granulocyte, macrophage, erythroid, megakaryocyte, and multipotential progenitors (2, 8–12). The predominant effect on neutrophil numbers observed in our model was not predicted by these data. An effect on monocyte and eosinophil production might have gone undetected if newly produced cells rapidly migrated from the circulation into tissues. The effect of GM-CSF on reticulocyte numbers (17) may depend on erythropoietin, a cofactor that was suppressed in our studies by red cell transfusion. The inconsistent effect of GM-CSF on platelet numbers might also reflect variable amounts of a second factor, e.g., thrombopoietin (22). The increase in platelet count observed after GM-CSF in the two animals with prolonged pancytopenia and bone marrow

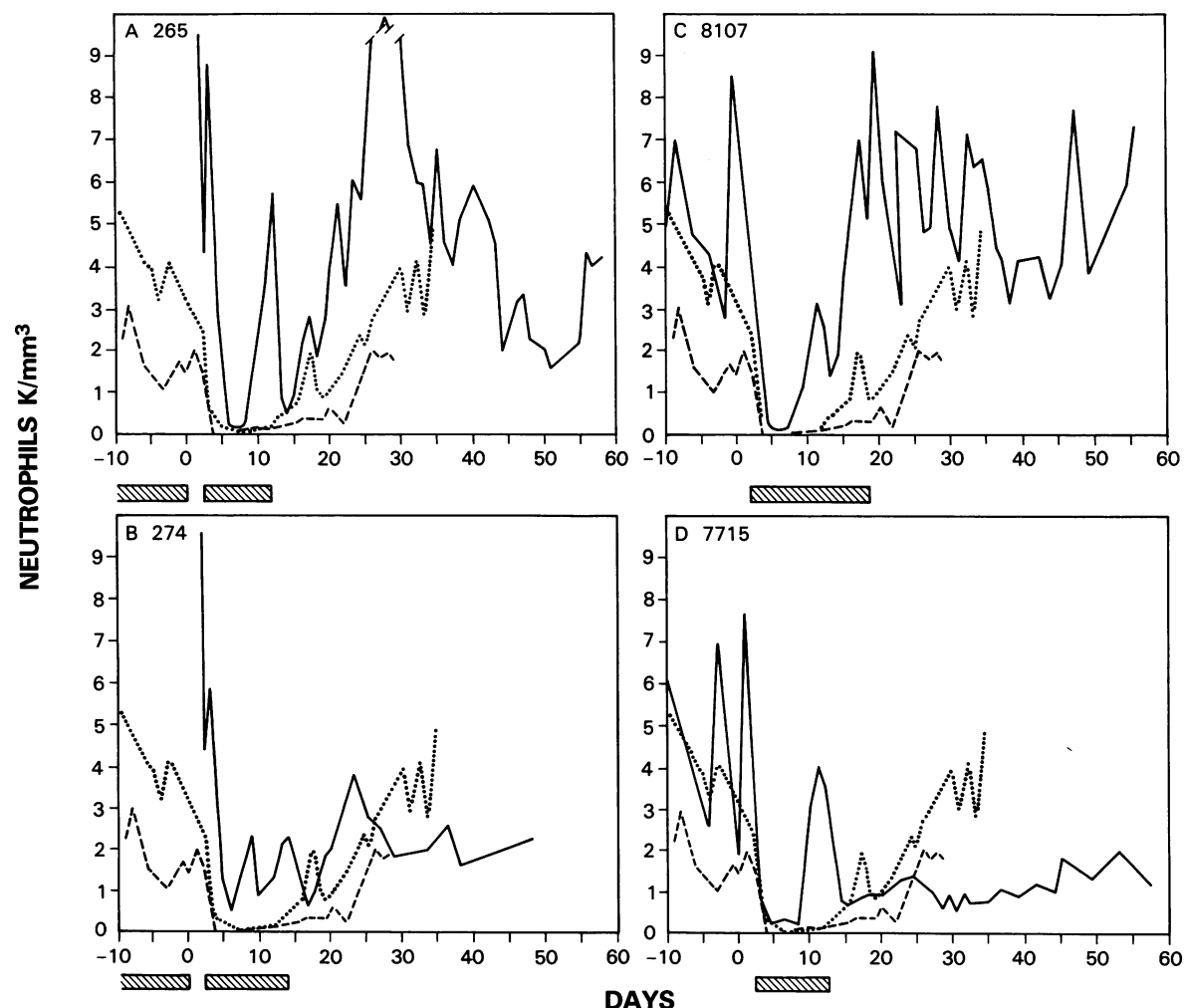


Figure 2. Recovery of neutrophil production in four animals treated with GM-CSF, two before and following bone marrow transplantation (A-265 and B-274) and two treated only following bone marrow transplantation (C-8107 and D-7715). Day 0 is the day of autologous bone

marrow transplantation. The period of GM-CSF administration is indicated below each graph. Data for the two control animals (0092---- and 829----) are also shown in each panel.

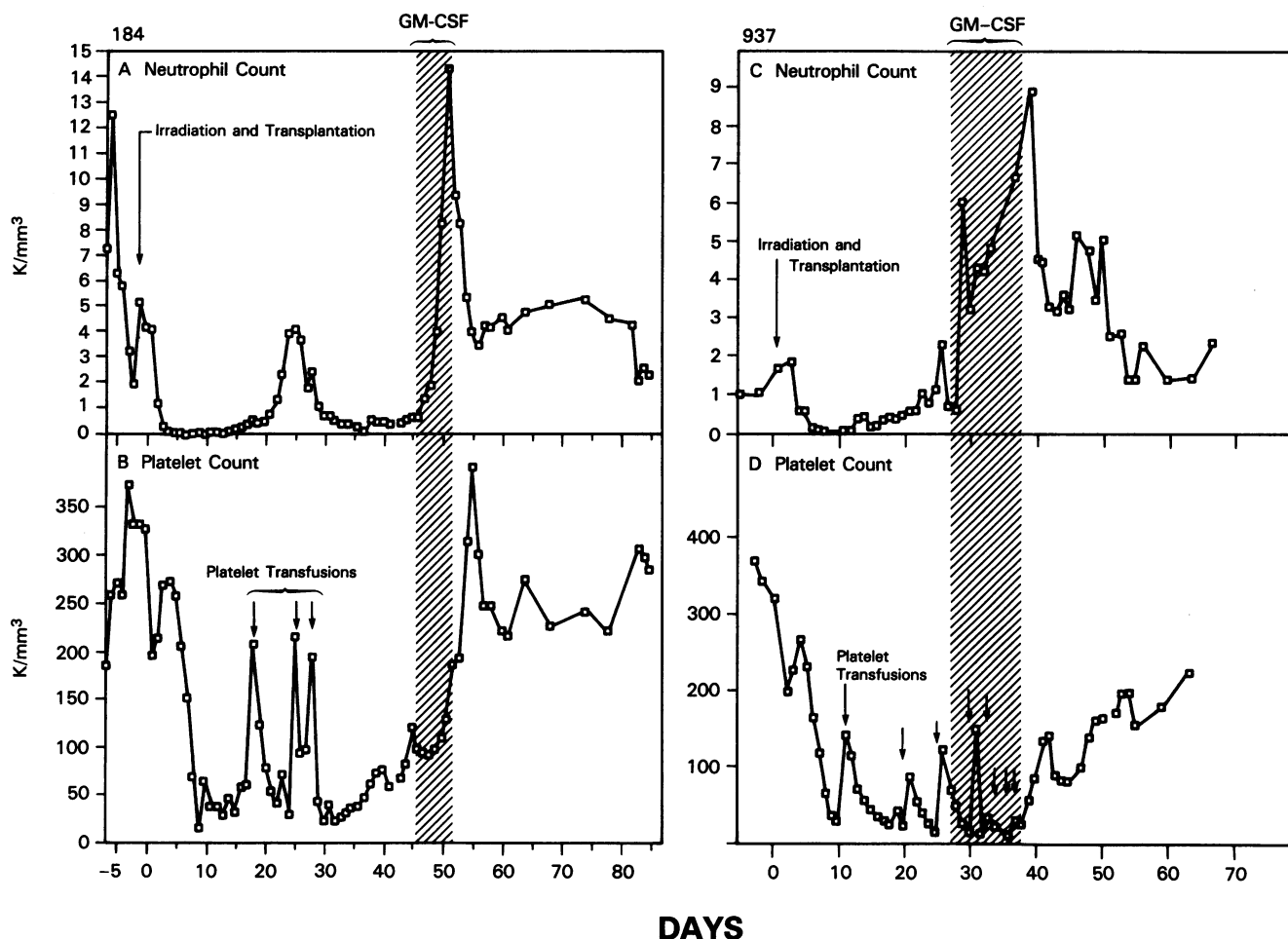


Figure 3. The effect of GM-CSF administration on neutrophil (A) and platelet (B) counts in two animals with delayed engraftment following autologous bone marrow transplantation. The period of GM-CSF administration is indicated by the cross-hatched area.

hypoplasia support an ability of this factor to augment platelet production.

The data suggest that GM-CSF acted on the bone marrow to increase neutrophil numbers. GM-CSF effects neutrophil migration and survival in vitro (23) and in vivo (unpublished observations). In normal animals, an increase in neutrophil count might rely in part on these effects but in our experiments, an increase in the neutrophil count was observed when animals were severely pancytopenic due to marrow depletion by irradiation. In vitro analysis has indicated that GM-CSF acts directly on progenitor cells through interaction with specific cellular receptors (24–26). The usual lag in production of neutrophils after marrow engraftment may reflect in part a local deficiency in hematopoietic growth factor production in the marrow microenvironment (16). This deficiency may be overcome by the high concentration of GM-CSF achieved by intravenous infusion. Delay or failure of marrow engraftment often occurs when T cells are removed from the graft in an effort to prevent graft vs. host disease (27). As T cells may be a major source of GM-CSF, administration of the factor might be particularly useful in such patients.

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