The Effects of Daily Recombinant Human Granulocyte Colony-Stimulating Factor Administration on Normal Granulocyte Donors Undergoing Leukapheresis

By William I. Bensinger, Thomas H. Price, David C. Dale, Frederick R. Appelbaum, Reginald Clift, Kathy Lilleby, Barbara Williams, Rainer Storb, E. Donnall Thomas, and C. Dean Buckner

The effects of daily administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF) to eight normal volunteers donating granulocytes for neutropenic relatives undergoing marrow transplantation were studied. Granulocyte donors consisted of seven marrow donors (5 syngeneic, 2 HLA identical) and one haploidentical son who had not donated marrow. All donors were administered daily rhG-CSF at a mean dose of 5 μg/kg/d (range 3.5 to 6.0) for a mean of 11.75 days (range 9 to 14 days), and granulocytes were collected a mean of 7.6 times (range 4 to 12). RhG-CSF was well tolerated and only minor side effects were observed. All donors became anemic from marrow donation and the removal of red blood cells during the collection procedures. Red blood cell transfusions were not given. All donors had a decrease in platelet counts and the magnitude of the decrement appeared to be greater than in historical donors. This was due in part to increased removal of platelets with the collection product, but a direct effect of rhG-CSF on platelet production cannot be excluded. The mean precollection granulocyte level was 29.6 × 10^9/L (range 11.8 to 79.8), which was a 10-fold increase over baseline. The mean number of granulocytes collected was 41.6 × 10^9 (range 1.3 to 144.1), which was a six-fold increase over historical donors not receiving rhG-CSF. The mean granulocyte level 24 hours after transfusion into neutropenic recipients was 0.95 × 10^9/L (median 0.57 and range .06 to 9.47). This study indicates that rhG-CSF is safe to administer to normal individuals, significantly improves the quantity of granulocytes collected, and results in significant circulating levels of granulocytes in neutropenic recipients. Further studies to evaluate rhG-CSF in normal granulocyte donors are warranted. © 1993 by The American Society of Hematology.

From the Fred Hutchinson Cancer Research Center, Seattle, WA.

DONORS AND METHODS

Eight normal adults (3 female, 5 male) volunteered to be granulocyte donors following the administration of rhG-CSF. Informed consent for rhG-CSF administration and granulocyte donation was obtained using a protocol approved by the institutional review boards of the University of Washington and the Fred Hutchinson Cancer Research Center (FHCRC). The protocol was also approved by the Food and Drug Administration and conducted with the use of an Investigational New Drug Application number BB-IND 4370. Seven granulocyte donors were marrow donors for their HLA-identical (2 cases), or syngeneic siblings (5 cases). One donated granulocytes to his HLA mismatched father who had received marrow from a different relative.

In the five syngeneic recipients, transfusions were given prophylactically beginning on day 1 or 2 after marrow transplant. In two recipients of allogeneic marrow, granulocyte transfusions were begun on the day after marrow infusion as therapy for documented fungal pneumonia and fungal septicemia respectively. In one allogeneic marrow recipient without engraftment transfusions were initiated on day 20 posttransplant for treatment of a bacterial cellulitis. All of the recipients were adults.

Immediately following marrow donation, seven donors received rhG-CSF (Amgen Inc, Thousand Oaks, CA) subcutaneously, which was administered daily thereafter. One donor had rhG-CSF initiated 19 days after marrow donation (1 day before granulocyte collection). The dose of rhG-CSF was 300 μg/d in five donors and 6 μg/kg body weight/d in the remaining three. In the five patients receiving the...
300 μg dose, the leukapheresis procedure took place 5 to 12 hours following the second and subsequent doses of rhG-CSF. In the three patients who received the 6 μg/kg dose, rhG-CSF was administered after each leukapheresis procedure. The study protocol provided for rhG-CSF administration for up to 14 days or until the recipient had pretransfusion granulocyte counts >1.0 × 10⁹/L on 2 consecutive days. If circulating granulocyte counts in the donor were >50 × 10⁹/L, rhG-CSF was omitted until levels were <50 × 10⁹/L.

Granulocyte collections were performed with an automated, continuous-flow blood cell separator (Cobe Spectra, Englewood, CO), processing 7 to 12 L of blood during a 3 to 4 hour period. In 5 donors, 30 mL trisodium citrate in 500 mL saline was used as the anticoagulant; these five donors received 200 to 800 mL of a pentastarch solution during the donation. In three donors, 400 mL saline with 100 mL hetastarch, 30 mL trisodium citrate concentrate, and 10,000 U sodium heparin was administered by continuous infusion during the leukapheresis procedure. A surgically placed right atrial catheter or a percutaneously placed subclavian catheter provided venous access. Complete blood cell counts including a 200 cell differential on white blood cells (WBC) were performed daily on all donors before and after collection and before the administration of rhG-CSF.

Collections from syngeneic donors were transfused immediately without irradiation. Collections from the allogeneic donors were irradiated with 25 Gy before transfusion. Recipient vital signs were monitored every 30 minutes during and for 1 hour following transfusion, and recipients of allogeneic cells were monitored with ear oximetry continuously during the infusion. All seven recipients given these transfusions early after marrow transplantation also received rhG-CSF (5 μg/kg/d) intravenously each morning until the granulocyte counts were >2 × 10⁹/L.

The number of granulocytes and other leukocyte types in blood and in collections were calculated from the WBC and differential counts (mature neutrophils plus band forms). For purposes of comparison, the experience with therapeutic granulocyte collections at the FHCRC between December 13, 1986 and July 14, 1991 was reviewed. During this time, granulocytes were collected from 13 normal donors for an average of 9.5 days (range 1 to 17) and transfused into 12 infected relatives after marrow transplantation. Two of the donors underwent two series of collections performed 1 month apart and 1 year apart. Seven of the 13 had also been marrow donors. Five were HLA identical with their recipients and two were mismatched for one HLA antigen. The remaining six granulocyte donors were HLA-haploidentical siblings or parents of the recipients. Two of these donors gave granulocytes for small children of <5 years of age. Six donors received 2 U or more of packed red blood cell transfusions during the course of the collections. None of these donors received stimulatory agents such as steroids or etiocholanolone. The Spectra was used for six series of collections and the 2997 (Cobe Laboratories, Englewood CO) was used for the other nine. All collections were performed using central venous catheters for blood access, 100 mL hetastarch, 30 mL trisodium citrate, and 10,000 U of sodium heparin added to 400 mL saline infused during the leukapheresis procedure. There were no significant differences between the granulocyte yields from either machine and so the data on yields were combined to form the historical control group.

STATISTICS

The differences in mean circulating granulocytes, hematocrits, and platelet counts over time between donors receiving rhG-CSF and historical donors were compared using a Wilcoxon rank-sum test. The mean total collections of granulocytes and platelets were compared using the Student’s t-test.

RESULTS

The eight donors in the study group received an average dose of 5 μg/kg rhG-CSF (range 3.5 to 6.0 μg/kg) administered for a mean of 11.75 days (range 9 to 14). Two donors had the day 12 dose of rhG-CSF omitted due to a granulocyte count of >50 × 10⁹/L, one donor received only nine doses because granulocyte collections were discontinued, and one donor failed to receive one scheduled dose.

Following the administration of rhG-CSF there was a rapid increase in peripheral WBC counts (Fig 1). The majority of cells circulating in the peripheral blood were mature neutrophils, but small numbers of bands, promyelocytes, metamyelocytes, and myelocytes began to appear in the circulation of most donors by day 4 after the start of rhG-CSF. The absolute numbers of lymphocytes increased slightly between days 4 and 12 while monocyte counts did not change. The granulocyte counts of donors receiving rhG-CSF were significantly higher than those of historical donors (P < .001) (Fig 2). Platelet counts decreased in the eight donors who received rhG-CSF. Platelet counts also decreased in the 13 donors who did not receive rhG-CSF but the decrement was significantly greater for donors who received rhG-CSF (P = .03). Hematocrit values also decreased in both groups but was greater for donors receiving rhG-CSF (P = .01). The initial decrease in hematocrit was due to blood loss during the marrow harvest. In the historical group packed red blood cells were transfused to six donors whose hematocrit decreased to <32%, while none of the donors receiving rhG-CSF were transfused.

Two donors experienced pain in the right femur and left elbow respectively on days 4 and 5, respectively. Their granulocyte counts were 31.4 × 10⁹/L and 21.2 × 10⁹/L, respectively at the time of the pain. The pain was mild, responded to acetaminophen, and lasted only 1 day in each donor. Fluid retention with weight gain attributed to pentastarch occurred in two donors. No other effects were observed, even when donors reached granulocyte levels >50 × 10⁹/L. No donor had to discontinue rhG-CSF for reasons other than for a granulocyte level >50 × 10⁹/L.

Donors receiving rhG-CSF underwent a mean of 7.6 collections (median 7, range 4 to 12). The quantity of granulocytes collected varied considerably from donor to donor and from day to day (Fig 3). The mean number of granulocytes collected from donors receiving rhG-CSF was greater than that collected from the unstimulated donors. The increase varied from threefold observed on days 1 to 3 to a 13-fold increase observed on day 9. At no time was the average yield of granulocytes <2.8 times that observed in unstimulated donors. Beginning on day 4 after the initiation of rhG-CSF, immature granulocyte precursors (promyelocytes, myelocytes, and metamyelocytes), began to appear in the collection products (Fig 3). Immature myeloid cells were not detected in collections from unstimulated donors. A summary of all collections is included in Table 1. Overall, a mean of six times more granulocytes were collected from donors receiving rhG-CSF than controls (P < .001).
The mean volume of packed red blood cells removed with each collection was 43.9 mL ± 20.8 in donors receiving rhG-CSF vs 43.0 mL ± 17.4 in the historical group. The average of the total numbers of platelets collected during each granulocyte harvest was $210 \times 10^9 \pm 166.9$ for donors receiving rhG-CSF compared with $140 \times 10^9 \pm 69.7$ in the historical group ($P = .001$). The mean volume collected was 471 mL ± 79.8 in the donors receiving rhG-CSF vs 354 mL ± 50.7 in donors in the historical group ($P < .001$).

The mean granulocyte levels in the peripheral blood of recipients 18 to 24 hours after each transfusion were calculated. Levels of granulocytes at 18 to 24 hours were compared with the historical experience with 13 patients who received therapeutic granulocyte transfusions. Peripheral blood granulocyte counts 18 to 24 hours after receiving transfusions from rhG-CSF–treated donors were significantly higher than observed in the historical group ($P < .001$). Mean granulocyte counts at 18 to 24 hours after transfusion averaged 5- to 10-fold higher each day in recipients of granulocytes collected following rhG-CSF. The overall mean granulocyte level was 19 times higher when patients received granulocytes from donors receiving rhG-CSF as compared with historical donors ($P < .001$) (Table 1). No adverse effects were observed in recipients who received granulocytes from donors administered rhG-CSF.

**DISCUSSION**

Previous collections of granulocytes from single donors by serial daily leukapheresis yielded low numbers of granulocytes with absent or very low 1-hour increments when transfused prophylactically into neutropenic patients.\(^\text{11,12}\) Daily granulocyte collections using a different donor each day yielded more granulocytes but the use of such donors increased the risk of alloimmunization\(^\text{13}\) and transmission of diseases such as cytomegalovirus.\(^\text{14}\) Improvements in apheresis techniques and stimulation of the donor with agents such as corticosteroids and etiocholanolone were of marginal benefit.\(^\text{15,16}\) For these and other reasons granulocyte transfusions are rarely used today. However, despite modern antibiotics, appreciable numbers of neutropenic patients are still encountered who have unresponsive bacterial or fungal infections. These patients could potentially have a benefit from granulocyte transfusions from normal donors.

In the present study rhG-CSF was administered to normal donors with minimal immediate side effects. However, the long-term effects of administering rhG-CSF to granulocyte recipients are unknown. Both the platelet count and hematocrit of donors receiving rhG-CSF decreased to levels significantly lower than in donors in our historical group. Platelet counts in the donors receiving rhG-CSF did not fall below the normal range, and it was considered safe to continue granulocyte collections. In patients receiving a 14-day course of rhG-CSF without prior chemotherapy, a dose-dependent reduction in platelet counts, occurring 2 to 4 days after the start of therapy and resolving approximately 12 days into therapy, has been reported by Lindemann et al.\(^\text{17}\) The decrease in platelets observed in our donors appeared due in part to the $50\%$ greater numbers of platelets in each granulocyte collection. It is unclear why more platelets were collected, although the $33\%$ larger volume of product collected undoubtedly accounts for some of the differences. The difference in volume could not, however, account for the six-fold greater numbers of granulocytes collected. The volume of packed red blood cells removed with each collection was the same in both groups. Since 6 of the 13 donors in the historical group received transfusions while none of the donors receiving...
rhG-CSF were transfused, the groups cannot be compared with regard to the hematocrit. The highest granulocyte level achieved by donors in this study was 70 to 80 × 10⁹/L. Levels below 50 × 10⁹/L were easily maintained by withholding doses of rhG-CSF. However, since donors reaching these levels were without symptoms, it may be safe to permit higher WBCs with or without higher doses of rhG-CSF resulting in a further increase in the
number of granulocytes collected. The increase in circulating granulocyte levels resulted in the collection of significantly greater numbers of granulocytes ($4.16 \times 10^9$) when compared with our historical experience ($6.8 \times 10^8$) or the experience using single donors administered steroids (10 to $20 \times 10^8$).

Collections following rhG-CSF contained immature granulocytes that were never observed in donors not receiving rhG-CSF. An increase of more immature myeloid forms in the peripheral blood after the administration of rhG-CSF to patients has been reported by several groups. Prevalent studies of prophylactic or therapeutic granulocyte transfusions into infected neutropenic recipients have failed to demonstrate sustained measurable granulocyte levels. Granulocyte levels at 1 hour rarely exceed $0.5 \times 10^9$ and very few, if any, granulocytes survived at 24 hours. In the present study granulocyte levels 18 to 24 hours after transfusion were higher in patients receiving granulocytes from rhG-CSF-treated donors than in historical patients. However, these results must be interpreted with caution. Five of the patients received prophylactic granulocyte transfusions from rhG-CSF-treated identical twin donors beginning when granulocyte counts fell below $0.5 \times 10^9$/L and it often required 1 to 2 more days before such patients become absolutely neutropenic. All recipients in the historical group received granulocytes for established infection, and consumption could have decreased the 24-hour granulocyte levels as compared with recipients receiving prophylactic granulocyte transfusions (5 of 8 patients in the present study). All of the recipients in the historical group received granulocytes from allogeneic donors compared with only three in the group receiving granulocytes from rhG-CSF-treated donors. Thus, alloimmunization could also have contributed to lower 24-hour granulocyte increments. However, it is just as likely that the higher circulating granulocyte levels in recipients of granu-
leucocytes from rhG-CSF–stimulated donors was due to the large cell dose and to the infusion of immature cells, which had a long half-life. It will obviously be of interest to determine in future studies if significant circulating levels of granulocytes can be maintained consistently in neutropenic recipients by transfusions from rhG-CSF–treated donors.

There is an extensive literature concerning the possible therapeutic or prophylactic effects of transfusing relatively small quantities of granulocytes into neutropenic individuals and this subject has been recently reviewed. Two major problems have been identified that have limited the evaluation of granulocyte transfusions, cell dose, and alloimmunization. The current data suggest that the problem of cell dose may be overcome with the use of recombinant growth factors such as the rhG-CSF. The use of rhG-CSF to boost donor circulating granulocyte levels may allow the selection and multiple use of HLA compatible donors, possibly avoiding alloimmunization or other immunologic complications. In addition, the transfusion of granulocytes from donors receiving rhG-CSF may convey additional benefits since studies have demonstrated that rhG-CSF–stimulated granulocytes have enhanced in vitro phagocytic and bactericidal activity. The markedly improved ability to mobilize and collect large numbers of granulocytes from normal donors should allow an evaluation of the effectiveness of transfused granulocytes in controlling refractory infections in granulocytopenic patients.

ACKNOWLEDGMENT

The authors are grateful to Mary Pettinger, MS, for assistance with the statistical analysis and to Kathy Knox for preparation of the manuscript.

REFERENCES

1. Karp JE, Merz WG, Dick JD, Saral R: Strategies to prevent or control infections after bone marrow transplants. Bone Marrow Transplant 8:1, 1991
15. McCredie KB, Freireich EJ, Hester JP, Vallejos C: Increased granulocyte collection with the blood cell separator and the addition of etiocholanolone and hydroxyethyl starch. Transfusion 14:357, 1974
The effects of daily recombinant human granulocyte colony-stimulating factor administration on normal granulocyte donors undergoing leukapheresis [see comments]

WI Bensinger, TH Price, DC Dale, FR Appelbaum, R Clift, K Lilleby, B Williams, R Storb, ED Thomas and CD Buckner