Effects of In Vivo Recombinant Methionyl Human Granulocyte Colony-Stimulating Factor on the Neutrophil Response and Peripheral Blood Colony-Forming Cells in Healthy Young and Elderly Adult Volunteers

By Gurkamal S. Chatta, Thomas H. Price, Robert C. Allen, and David C. Dale

Recombinant granulocyte colony stimulating factor (G-CSF) was administered daily for 14 days to healthy young (Y) (20 to 30 years) and elderly (E) (70 to 80 years) volunteers to evaluate the effects of age on the neutrophil (polymorphonuclear leukocytes, PMN) responses. Thirty-eight volunteers were randomized to receive 0 pg, 30 pg, or 300 pg per day. Baseline neutrophil counts (ANC), peak ANCs, and the rate of attaining the peak ANC were similar in both age groups at both doses. The peak ANC was increased 5-fold at 30 pg and 15-fold at 300 pg in both the young and elderly. Daily tests of PMN function, as measured by an automated chemiluminescence system, showed nearly identical responses to several agonists for both age groups. Marrow proliferative activity as reflected by the percentage of cells in the marrow neutrophil mitotic pool also increased similarly for both age groups at both doses. In contrast, there was an age-related change in blood colony formation as measured by the blood CFU-GM assay. Compared with controls at the 30 pg dose, mean colony formation was increased 2-fold in the young versus no change in the elderly and at the 300 pg dose 24-fold in the young versus 12-fold in the elderly. These studies indicate that neutrophil responses to rhG-CSF are equivalent in healthy young and elderly volunteers but the mobilization of progenitor cells, as measured by the CFU-GM assay appears to differ substantially.

NEUTROPENIA caused by chemotherapy-related myelosuppression1 or with severe infections2,3 is an important clinical problem in the elderly. The physiologic basis of this suboptimal neutrophil response remains unclear, but it is associated with a poor prognosis in cancer patients and in many infectious diseases.4,5 At the cellular level, this defect in myelopoiesis has been attributed to age-related deficits in marrow progenitor cell numbers,6 changes in the marrow microenvironment,7 decreased production of regulatory growth factors,8 or a combination of these mechanisms.9,10 Recently, we have shown that marrow progenitor numbers are preserved with aging, but there is a diminution in the in vitro sensitivity of marrow myeloid precursors to granulocyte colony-stimulating factor (G-CSF).11

G-CSF is a growth factor with the capacity to promote the growth and maturation of myeloid cells and, in particular, the proliferation and differentiation of neutrophil progenitors both in vitro and in vivo.12 Phase III clinical trials have established the safety and efficacy of recombinant human G-CSF (rhG-CSF) in ameliorating chemotherapy-related neutropenia in cancer patients and for the treatment of severe chronic neutropenia.13-15 More recently, rhG-CSF has also been used for mobilizing stem cells into peripheral blood (PBSC) in a dose-dependent fashion for autologous bone marrow transplantation.16-20 However, there is little information on the effects of rhG-CSF on the blood and marrow polymorphonuclear leukocyte (PMN) response in normal persons and the influence of age on this response. In this report we compare changes in PMN numbers and function and changes in peripheral blood colony forming cells (PB-CFU-GM) in healthy young and healthy elderly volunteers following the subcutaneous administration of rhG-CSF.

MATERIALS AND METHODS

Volunteers

Nineteen young (Y) (age 20 to 30 years) and nineteen elderly (E) (age 70 to 80 years) volunteers of both sexes were recruited from the community in accordance with the protocols approved by the Human Subjects Review Committee of the University of Washington and the Clinical Research Center at the University of Washington Medical Center, Seattle. All the volunteers were nonsmokers, were on no prescription medications, had normal physical examinations, and had had no acute illness in the 6 weeks preceding the study. The volunteers also had a normal CBC, urinalysis, and renal, liver, and thyroid function tests. Informed consent was obtained before the study.

Study Design

This was a phase I, randomized, open-label, controlled study. Volunteers fulfilling all the eligibility criteria were randomized to one of three dosage groups: no drug (5 Y, 5 E), 30 pg rhG-CSF (7 Y, 7 E), and 300 pg rhG-CSF (7 Y, 7 E). The total study period for each subject was 17 days: a 2-day baseline evaluation period followed by a 15-day testing period (with or without 14 days of rhG-CSF, depending on the randomization assignment). The entire study was conducted between July 1991 and June 1993.

Throughout the 15-day testing period, subjects were monitored with daily vital signs, CBC and measurements of neutrophil chemiluminescence. Bone marrow aspirates were obtained on days 0, 5, and 15. PB-CFU-GM were evaluated both prior to and 5 days following administration of rhG-CSF. All injections of rhG-CSF (Amgen, Thousand Oaks, CA) were given subcutaneously between 7 AM and 9 AM daily. A record of all concomitant medications and nutritional supplements taken during the study period was maintained. The use of the following medications was specifically excluded: nonsteroidal anti-inflammatory agents (including aspirin), H2 receptor antagonists, antimicrobials, corticosteroids (including topical agents), and lithium. At the end of the 15-day testing period, all volunteers had an exit physical examination, urinalysis, and blood for CBC with reticulocyte count and a routine chemistry panel.

From the Department of Medicine, University of Washington, Puget Sound Blood Center, Seattle, WA, and ExOxEmis, Inc, San Antonio, TX.

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Address reprint requests to Gurkamal S Chatta, MD, Department of Medicine, RG-22, Health Science Bldg, AA522, University of Washington, Seattle, WA 98195.

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Measurements

CBC. Using standard techniques, CBCs were performed at 8 AM each day (before administration of rhG-CSF) on 2 mL EDTA anticoagulated samples with a Coulter counter (Hialeah, FL), and 200 cell manual differentials. Additional CBCs were performed at 12 hours after rhG-CSF administration on study days 9 to 14. Reticulocytes were measured on days 0 and 15.

Bone marrow aspirates. Two milliliters of marrow was aspirated from the posterior superior iliac crest on days 0 and 5, and in some subjects on day 15. The marrow smears were evaluated for differential counts, PMN maturation, and myeloid to erythroid ratios. The cumulative number (percentage) of myeloblasts, promyelocytes, and myelocytes was used to represent the marrow mitotic pool and the sum of metamyelocytes, bands, and PMNs was taken to represent the marrow postmitotic pool.

Neutrophil function using an automated luminescence system. The respiratory burst metabolism of circulating PMNs was measured using an automated chemiluminescence system and reagents (the AXIS²; ExOxEmis Inc, San Antonio, TX), as previously described.¹¹ The AXIS² reagents included (1) blood diluting medium (BDM), (2) dimethylbiacridinium (DBA⁺⁺) nitrate balanced salt solution (DBSS), (3) luminol balanced salt solution (LBSS), and (4) prefabricated tubes coated with phosphor myristate acetate (PMA) to directly stimulate respiratory metabolism. The use of activity-specific chemiluminescent substrates allowed differential measurement of PMN respiratory burst activities. Reductive oxygenation (RDOX) of DBA⁺⁺ yields luminescence, which is dependent on the generation of superoxide by NADPH oxidase. Simple oxygenation (DOX) of luminol can be catalyzed by myeloperoxidase and other mechanisms.

Whole blood (0.1 mL) was diluted in BDM (9.9 mL) and loaded into the luminometer (Berthold LB953 AXIS-modified, Wildbad, Germany). Chemically stimulated RDOX activity was measured automatically after luminometer injection of 0.1 mL of diluted blood (ie, 1 μL equivalent of whole blood) into tubes containing PMA (10 pmol/L) with 0.6 mL of DBSS as substrate medium.

DOX activity was measured using high-dose PMA (5 pmol/L) with 0.6 mL of LBSS as substrate medium. The 10 pmol/L PMA tubes contain sufficient stimulus to produce specific degranulation with oxidase activation and the 5 pmol/L PMA tubes have sufficient stimulus to produce specific plus azurophilic degranulation. Luminescence measurements were made in triplicate over a 20-minute interval. The results were expressed as the specific luminescence activity per PMN, ie, counts/20 min/PMN.⁰² Oposin receptor-dependent DOX was also measured. Maximum oposin receptor expression per PMN was elicited using human recombinant C5a coated tubes (2.5 pmol/L) and platelet activating factor (PAF) coated tubes (1.2 nmol/L). These quantities of C5a and PAF are sufficient to induce maximum oposin receptor expression per neutrophil; ie, CD11b/CD18 and CD35, expression without producing large increases in respiratory burst metabolism. The oposin stimulus was human complement-opsinized zymosan (hC-opZ) and the substrate medium was 0.6 mL of LBSS.⁰²

PB-CFU-GM. Colony formation from peripheral blood samples was assayed in vitro before rhG-CSF treatment (day 0) and on days 1 and 5, at 5 hours after administration of rhG-CSF in a serum-containing system using established methods.⁰² Briefly, 25 mL of heparinized venous blood was diluted 1:1 with media (NCTC), underlayered with ficoll-hypaque and centrifuged at 1,300 rpm for 45 minutes at room temperature. The mononuclear cells (MNCs) obtained were washed three times in media, counted, and then resuspended in 10% fetal calf serum (FCS) in NCTC at a concentration of 5 × 10⁶ MNCs/well. One milliliter containing 5 × 10⁵ MNCs with 20% FCS, 0.3% agar (Difco Lab, Detroit, MI), and NCTC, both with and without a combination of cell stimulants [rhG-CSF (282 pmol/L) + rhGM-CSF (50 pmol/L) + rhlL-3 (40 pmol/L)], was plated onto 35-mm petri dishes ( Falcon Lab, Lincoln Park, NJ) and placed in a 37°C, 5% CO₂, 100% humidity incubator. All assays were performed in triplicate and colonies (>50 cells) were scored with an inverted microscope on day 14. The results were expressed as number of colonies per milliliter of blood. For colony differentials, the colony forming units (CFUs) in the agarose were transferred to glass slides, fixed with 5% glutaraldehyde, and then Giemsa stained.

Statistical Analysis

Student's t-test and 1 standard deviation confidence interval were used to analyze the data unless otherwise specified. Because the data on the colony formation were positively skewed, a log transformation was performed to stabilize the variances in the data. Next, a three-dose level by two age group analysis of variance (ANOVA) was performed on the log transformed colony counts.

RESULTS

Volunteers

A total of 39 healthy young (mean age 23 years) and healthy elderly (mean age 74 years) volunteers of both sexes were recruited, and all but one volunteer completed the study. A single dropout from group Y occurred 3 days into the study due to personal reasons. There were no differences in the baseline hematologic parameters between the young and elderly subjects (Table 1). Other than mild bone pain, no volunteers developed significant side effects. Exit physical exams and repeat blood chemistries were normal with one exception; one volunteer from group O had a transient elevation in levels of uric acid and liver function tests, without associated symptoms. Compared with baseline, there were no significant changes in the median hematocrits, platelet counts, or the reticulocyte counts at the completion of the study for either the young or elderly subjects.

Neutrophil Response

The baseline PMN counts were similar in the two groups: 3,149 ± 349/mm³ in group Y versus 3,601 ± 342/mm³ in group O. In both the groups, the PMN counts peaked and plateaued at day 8 to 9 (Fig 1). At both dosages of rhG-CSF, the augmentation in the PMN count was consistently twofold higher at 8 PM, compared with the 8 AM levels (Fig 2). There was no significant age-related difference (P > 0.05) in the overall PMN response between the two groups for

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All values expressed as mean ± 1 SEM.
AGING, rhG-CSF, AND BLOOD AND MARROW RESPONSE

Response to rhG-CSF

Fig 1. PMN counts in young (○) and elderly (□) volunteers administered daily subcutaneous rhG-CSF (0 μg, 30 μg, and 300 μg) over a 15-day period. All counts were made daily at 8 AM. Each data point represents the mean PMN count ± 1 SEM.

either the morning or evening counts. The mean PMN counts for the elderly subjects for study days 2 through 5 were significantly higher (P = .03) than for the young subjects; thereafter they were not different (P = .4). The peak PMN counts in the young and the elderly were 18,106 ± 2,231/mm³ versus 15,934 ± 2,214/mm³ at the 30 μg dose (5-fold increases) and 45,675 ± 4,976/mm³ versus 52,944 ± 5,934/mm³ at the 300 μg dose (15-fold increases), respectively (Fig 2). Immediate neutrophil response to rhG-CSF (PMN count 5 hours after administration of drug) was also equivalent at both 30 μg and 300 μg of G-CSF in the two groups (data not shown). At the 30 μg dose, 4% to 5% of the circulating cells were bands and at the 300 μg dose, there were 14% to 15% circulating bands in both the young and the elderly. In contrast, the control group (0 μg rhG-CSF) had <1% circulating bands.

Bone Marrow Changes

There were no differences in marrow differential counts for the two age groups before treatment. The most pro-
nounced changes in marrow morphology occurred at the 300-μg dose on day 5 (Table 2). There was a 2.5-fold increase (P < .001) in the percentage of marrow mitotic cells (promyelocytes + myelocytes) and a slight decrease (P = .18) in the percentage of marrow postmitotic cells (metamyelocytes + bands + neutrophils) on day 5. By day 15, the M/E ratio had increased significantly (P < .01) and the percentage of postmitotic cells of the neutrophilic series was similar to day 0 (data not shown). At the 30-μg dose on day 5, there was a significant increase in the marrow mitotic cells (P < .01) and no change in the post-mitotic cells. These changes in marrow differential counts were similar for both age groups (Table 2).

PMN Metabolic Function

Age had no significant influence on neutrophil respiratory burst metabolism before or after rhG-CSF treatment. As shown in Fig 3A, rhG-CSF at both the 30 μg and the 300 μg dose levels did not increase the specific oxidase (ie, RDOX) activities in either age group. However, both rhG-CSF–treated groups (Y and O) showed substantial increases in PMA-stimulated DOX activities (myeloperoxidase-dependent) at both dose levels. As shown in Fig 3B, the myeloperoxidase-dependent activity at day 5 increased approximately 2.5-fold at the 30 μg dose (P < .01) and 5-fold at the 300 μg dose (P < .001). The oxidase and myeloperoxidase activities of the control group (no rhG-CSF) were unchanged throughout the study. Compared with receptor-independent activation, treatment with rhG-CSF had only a modest effect on opsonin receptor-dependent activation of DOX (myelo-}

| Table 2. Bone Marrow Differential Counts Before and After Five Days of rhG-CSF |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                         | Young                    | Old                      | Must                          |
|                         | Day 0 Day 5              | Day 0 Day 5              |                          |
|                         | (n = 6) (n = 6)          | (n = 6) (n = 6)          |                          |
| Myeloblasts             | 0.4 ± 0.1                | 0.7 ± 0.2                | 0.5 ± 0.1                  |
| Promyelocytes           | 3.1 ± 0.4                | 7.2 ± 1.1                | 2.4 ± 0.2                  |
| Myelocytes              | 9.8 ± 1.4                | 17.1 ± 1.3               | 11.7 ± 0.1                |
| Metamyelocytes          | 10.8 ± 0.8               | 9.2 ± 0.7                | 10.8 ± 1.4                |
| Bands                   | 9.9 ± 1                  | 8.5 ± 0.7                | 11.0 ± 0.9                |
| PMNs                    | 12.8 ± 1.6               | 14.2 ± 1.8               | 12.1 ± 1.2                |
| M/E Ratio               | 1.6 ± 0.2                | 2.3 ± 0.5                | 1.7 ± 0.2                  | 1.9 ± 0.4                  |
|                         | 300 μg rhG-CSF           |                          |                          |
|                         |                         | Day 0 Day 5              | Day 0 Day 5              |
|                         |                         | (n = 7) (n = 7)          | (n = 7) (n = 7)          |
| Myeloblasts             | 0.6 ± 0.2                | 0.4 ± 0.1                | 0.5 ± 0.1                  |
| Promyelocytes           | 2.8 ± .3                 | 7.1 ± 0.3                | 3.2 ± 0.5                  |
| Myelocytes              | 9.7 ± 0.9                | 21.9 ± 2.2               | 12.1 ± 0.6                |
| Metamyelocytes          | 11.2 ± 1.3               | 14.1 ± 1.3               | 12.0 ± 0.3                |
| Bands                   | 13.2 ± 1.3               | 12.8 ± 2.3               | 14.0 ± 0.5                |
| PMNs                    | 13.9 ± 1.8               | 6.7 ± 1.3                | 12.0 ± 0.2                |
| M/E Ratio               | 1.9 ± 0.3                | 3.3 ± 1.3                | 2.2 ± 0.4                  | 3.8 ± 0.4                  |

Fig 3. Effect of rhG-CSF on (A) receptor-independent oxidase activity and (B) receptor-independent myeloperoxidase activity. Reductive dioxygenation of dimethylbenzidine balanced salt solution (DBSS) in the presence of PMA results in luminescence that is oxidase-dependent (A), and simple dioxygenation of luminol balanced salt solution (LBSS) in the presence of PMA results in luminescence that is primarily myeloperoxidase-dependent (B). The PMA-stimulated activities were pooled for the 0 μg dose and reported as mean count ± 1 SD for Y and O. For the 30-μg and 300-μg dosages, the mean oxidase activity (A) and the mean myeloperoxidase activity (B) in each group on days 5 and 9 are shown.

peroxidase-dependent). C5a and PAF were used to prime maximum opsonin receptor expression (MORE) so that exposure to a non–rate-limiting quantity of opsonin, ie, hC-OpZ, yields maximum phagocytosis and respiratory metabolism. Table 3 presents the C5a and PAF-dependent DOX activities of the 30 μg and 300 μg rhG-CSF groups pretreatment and on days 5 and 9 of the study. Compared with PMA DOX activities, rhG-CSF produced only modest increases in MORE DOX activities. At day 5, the MORE DOX activities were increased only 2-fold for the 300 μg rhG-CSF group (P > .01), but by day 9 the response was essentially the same as for the 30 μg rhG-CSF group.

Peripheral Blood Colony Formation (CFU-GM)

In both the young and the elderly, spontaneous CFU-GM formation and colony formation stimulated in vitro by the combination of G-CSF, GM-CSF, and interleukin 3 (IL-3), were evaluated on day 0, and 5 hours after administration of rhG-CSF on days 1 and 5. In both age groups, there was
minimal spontaneous colony formation on days 0 and 1 (15 ± 9 colonies/mL) and stimulated colony formation on days 0 and 1 was similar at all dosages (33 ± 11 colonies/mL). However, there was both a dose-dependent and an age-dependent change in blood CFU-GM on day 5. Compared with the 0 μg group, spontaneous colony formation on day 5 was increased 3-fold in the young (46 ± 22 cols/mL) (P = .17) but was unchanged in the elderly (10 ± 5 cols/mL) at the 30 μg dose; at the 300 μg dose, spontaneous colony formation was increased 12-fold in the young (174 ± 72 cols/mL) (P = .09) and 16-fold in the elderly (233 ± 106 cols/mL) (P = .07). Stimulated colony formation on day 5 was increased 2-fold (68 ± 31 cols/mL) at the 30 μg dose (P = .08) and 24-fold (790 ± 230 cols/mL) at the 300 μg dose (P = .02) in the young. There was no change (36 ± 11 cols/mL) at the 30 μg dose and a 12-fold increase (411 ± 105 cols/mL) at the 300 μg dose (P = .01) in the elderly (Fig 4). The mean colony number at the 300 μg dose was twofold higher in the young (790 ± 230 in the young vs 411 ± 105 in the elderly). Because these data were skewed, further analysis of the response at 300 μg were made after logarithmic transformation of the colony counts. The two factor ANOVA found a large dose effect for the day 5 stimulated colony formation, F (2,34) = 25.04; P = .0000. There was also a significant age effect, F (1,34) = 5.06; P = .032, with the response of group 0 being 60% less than group Y across all dose levels. Spontaneous colony formation at day 5 also showed a significant dose effect, F = 9.59; P = .0006, but failed to detect any age effect.

**DISCUSSION**

We examined the effects of subcutaneously administered rhG-CSF at two dosages, 30 μg/d and 300 μg/d (approximately 0.5 μg/kg/d and 5 μg/kg/d) in healthy young and healthy elderly subjects over a 2-week period. At these dosages, rhG-CSF was well tolerated in both the groups with an equivalent increase in PMN responses both in the marrow and blood. There was also no age-related difference in PMN function as measured by chemiluminescence. However, there was a statistically significant age-dependent decrease in the ability to mobilize PB-CFU-GM with rhG-CSF treatment, the response being twofold less in the elderly.

The increase in PMNs in blood was dose dependent (5-fold at 30 μg and 15-fold at 300 μg) and substantially higher PMN levels were consistently observed 12 hours after rhG-CSF. Both the magnitude and the timing of the PMN increase is consistent with results from other clinical trials and the available information on the pharmacokinetics of subcutaneous administration of rhG-CSF. Our study extends these observations to normal subjects and also suggests that at least in the healthy elderly, in accordance with a recent report, there is no compromise either in the magnitude or in the timing of the PMN response to rhG-CSF. Previously, endotoxin and hydrocortisone have been shown to elicit a subnormal PMN response in the elderly. Our data suggest that these agents cause neutrophilia by different mechanisms and that the mode of action of endotoxin and hydrocortisone is in some way susceptible to the aging process. Endotoxin administration can markedly elevate endogenous G-CSF levels but the effects of hydrocortisone on G-CSF levels have not been reported. Aging may impair the ability of these agents to upregulate levels of G-CSF or other cytokines involved in this response. However, available data indicate that at least with severe infections, G-CSF levels can increase to a comparable degree in young and elderly patients. Finally, as evidenced by the lack of bands in the

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<th>Table 3. Effect of rhG-CSF on Opsonin Receptor Expression</th>
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PMNs were isolated daily from peripheral blood, and maximum opsonin receptor expression per PMN was elicited using human re-combinant C5α-coated tubes (2.5 nmol/Ltube) and platelet-activating factor (PAF)-coated tubes (1.25 nmol/Ltube) in an automated chemiluminescence system. These quantities of C5α and PAF are sufficient to induce maximum opsonin receptor expression without producing large increases in respiratory burst metabolism. The opsonic stimulus was human complement opsonized zymosan and the chemilumigenic substrate was luminol balanced salt solution.

Fig 4. Peripheral blood colony formation (PB-CFU-GM) 5 days after administration of rhG-CSF. Mononuclear cells were plated with (stimulated) and without (spontaneous) a combination of growth factors (rhG-CSF, rhGM-CSF, and IL-3). Both spontaneous and stimulated PB-CFU-GM were quantitated on day 14, with all assays being performed in triplicate. The results are expressed as number of colonies/mL of blood ± 1 SEM. (□), Spontaneous, 0 μg; (■), stimulated, 0 μg; (□), spontaneous, 30 μg; (□), stimulated, 30 μg; (□), spontaneous, 300 μg; (■), stimulated, 300 μg.

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CSF caused large increases in high-dose PMA-activated DOX activity (receptor-independent). It is interesting that fully elicited, for the dosages used in this study, rhG-pathway is well preserved in the healthy elderly. In independent (tested with PMA) pathways. Although the preliminary inhibition studies and direct measurements of PMNs to respond to opsonin presented in excess can provide PMN without causing significant activation of respiratory oxidase-driven myelocyte stage with no significant change in marrow myeloblasts. Hence, G-CSF induced neutrophilia can in large part be accounted for by increased PMN production and a shortened PMN maturation time in the marrow. This, together with previous data showing the lack of cycling of marrow progenitors in patients treated with rhG-CSF, would suggest that G-CSF expands the marrow myeloid pool at or distal to the CFU-GM stage and that this portion of the myeloid pathway is well preserved in the healthy elderly.

The effects of in vivo rhG-CSF on PMN function as measured by chemiluminescence were also similar in the two age groups. PMN microbicidal function is dependent on the circulating phagocyte’s capacity to respond to activation signals and to produce the oxygenation reagents required for microbe killing. PMN activation occurs both via opsonin receptor-dependent (tested with PAF and C5a) and receptor-independent (tested with PMA) pathways. Although the precise effects of G-CSF on PMN function have not yet been fully elucidated, for the dosages used in this study, rhG-CSF caused large increases in high-dose PMA-activated DOX activity (receptor-independent). It is interesting that PMA-activated DOX was maximal around day 5. This increased DOX activity suggests a temporal linkage in that the day 5 PMNs would have been initially exposed to rhG-CSF when their precursors were in the marrow mitotic pool. Although DOX activity primarily reflects oxidase-driven myeloperoxidase activity in normal untreated subjects, our preliminary inhibition studies and direct measurements of myeloperoxidase activity per PMN suggest that the fivefold increase in DOX cannot be explained by increased myeloperoxidase alone. Relatively low concentrations of C5a and PAF, can induce maximum opsonin receptor expression per PMN without causing significant activation of respiratory burst metabolism. The capacity of opsonin receptor primed PMNs to respond to opsonin presented in excess can provide a functional evaluation of the opsonin-opsonin receptor ligation process responsible for activation of PMN metabolism. Although treatment with rhG-CSF also increased opsonin receptor-dependent activation of DOX, the increases observed were modest in comparison with PMA-stimulated DOX activity. Previous reports on aging and PMN function are conflicting. Our data clearly indicate that RDOX, PMA-stimulated DOX and opsonin-stimulated DOX activities are similar in the young and elderly both before and with rhG-CSF treatment.

The reduced ability of the elderly to mobilize PB-CFU-GM in response to rhG-CSF was the only age-related change observed. The differences in colony formation at both dosages were statistically significant when analyzed by ANOVA, with colony formation in the elderly being 60% less than the young across all dose levels. Because the age-related difference in colony formation was of a somewhat smaller magnitude at the higher dose of G-CSF, it is possible that at doses of G-CSF exceeding those used in this study, i.e., greater than 5 µg/kg, the young and the elderly may mobilize PB-CFU-GM to a comparable level. This would be consistent with our previous work, which showed that bone marrow hematopoietic progenitors from the healthy elderly have reduced sensitivity, but similar absolute responsiveness to G-CSF compared with healthy young subjects. Although the biologic characteristics of PBSC are not completely known, their mobilization with rhG-CSF and subsequent use for hematopoietic reconstitution is now established.

Our results would suggest that higher doses of rhG-CSF may need to be used in the elderly to mobilize PBSC. The concern about hematopoietic exhaustion with aging remains unanswered. But from available data in animals, the proliferative capacity of hematopoietic stem cells, though finite, is thought to be well in excess of the life-span of a species.

In conclusion, this study examining the effects of age on the blood and marrow responses in healthy volunteers to the administration of rhG-CSF shows that the PMN responses from the myeloblast to the blood PMN are not compromised with age and that G-CSF is well tolerated by normal volunteers of both age groups, without significant side effects. Based both on our previous work, as well as the results of the present study, there is probably the existence of important age-related deficits in the early stages of hematopoiesis. These deficits are likely to be clinically significant either when present cumulatively or under conditions of hematopoietic stress. Further efforts in aging research related to granulocytopenia should focus on defining the basis for these changes.

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REFERENCES

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42. Chervenick PA, Boggs DR: In vitro growth of granulocytic and mononuclear cell colonies from blood of normal individuals. Blood 37:131, 1971


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Effects of in vivo recombinant methionyl human granulocyte colony-stimulating factor on the neutrophil response and peripheral blood colony-forming cells in healthy young and elderly adult volunteers

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