Recombinant Rat Stem Cell Factor Synergizes With Recombinant Human Granulocyte Colony-Stimulating Factor In Vivo in Mice to Mobilize Peripheral Blood Progenitor Cells That Have Enhanced Repopulating Potential

By R.A. Briddell, C.A. Hartley, K.A. Smith, and I.K. McNiece

Splenectomized mice treated for 7 days with pegylated recombinant rat stem cell factor (rrSCF-PEG) showed a dose-dependent increase in peripheral blood progenitor cells (PBPC) that have enhanced in vivo repopulating potential. A dose of rrSCF-PEG at 25 μg/kg/d for 7 days produced no significant increase in PBPC. However, when this dose of rrSCF-PEG was combined with an optimal dose of recombinant human granulocyte colony-stimulating factor (rhG-CSF; 200 μg/kg/d), a synergistic increase in PBPC was observed. Compared with treatment with rhG-CSF alone, the combination of rrSCF-PEG plus rhG-CSF resulted in a synergistic increase in peripheral white blood cells, in the incidence and absolute numbers of PBPC, and in the incidence and absolute numbers of circulating cells with in vivo repopulating potential. These data suggest that low doses of SCF, which would have minimal, if any, effects in vivo, can synergize with optimal doses of rhG-CSF to enhance the mobilization of PBPC stimulated by rhG-CSF alone.

AUTOTOLOGOUS bone marrow transplantation (BMT) has been used for support of high-dose chemotherapy in the treatment of a number of neoplastic diseases. Recent studies have shown enhanced engraftment of peripheral blood progenitor cells (PBPC) compared with BMT in this setting.1 Cytotoxic chemotherapy and/or cytokine administration has been used to increase the number of circulating white blood cells (WBC), cells expressing the surface antigen CD34 (CD34+), and granulocyte-macrophage colony-forming cells (GM-CFC).2,3 In particular, treatment with recombinant human granulocyte colony-stimulating factor (rhG-CSF) mobilizes PBPC that give rise to more rapid neutrophil and platelet engraftment.4,5

Recently, Andrews et al6 have shown that treatment of normal baboons with recombinant human stem cell factor (rhSCF) leads to increased numbers of PBPC, consisting of both primitive (high proliferative potential–colony-forming cell [HPP-CFC]) and mature (GM-CFC) progenitor cells.6 When used to reconstitute lethally irradiated baboons, PBPC from rhSCF-treated baboons led to long-term survival for 2 of 3 animals, whereas 4 of 4 animals receiving transplants of the same number of PBPC from untreated baboons failed to survive.7

In this study, we have examined the ability of pegylated recombinant rat stem cell factor (rrSCF-PEG) to mobilize PBPC in mice and have determined the repopulating potential of these cells in lethally irradiated mice. We have also compared rrSCF-PEG–mobilized PBPC with rhG-CSF–mobilized PBPC. Finally, we have explored the potential of PBPC mobilization using the combination of rrSCF-PEG and rhG-CSF.

MATERIALS AND METHODS

PBPC Mobilization

Four-week-old male (C57Bl/6J X DBA/2J) F1 mice (Charles River Laboratories, Wilmington, MA) were splenectomized and housed under sterile conditions until they reached 10 weeks of age. The mice were injected intravenously with rrSCF-PEG, rhG-CSF, or the combination of both cytokines daily for 7 days. Each experiment also contained a group of mice treated only with the carrier, consisting of 1% bovine serum albumin (Sigma Chemical Co, St Louis, MO) suspended in isotonic saline.

Cell Harvesting

Peripheral blood was harvested by cardiac puncture and collected into EDTA-containing tubes. Bone marrow cells were harvested by flushing the contents of the femur with 1 mL of Hanks' Balanced Salt Solution (HBSS; Gibco Laboratories, Life Technologies, Inc; Grand Island, NY) containing 2% fetal bovine serum (Flow Laboratories, Inc, McLean, VA). The numbers of WBC, red blood cells (RBC), and platelets in the peripheral blood were determined using a Sysmex Microcellcounter (Baxter Healthcare Corp, Irvine, CA). Bone marrow WBC numbers were determined by staining with crystal violet and subsequently enumerated on a hemacytometer.

Peripheral Blood Density Gradient Fractionation

Peripheral blood was pooled from all mice in each treated group and layered over single Nycojen density gradients containing 1.167 g/mL (Accurate Chemical and Scientific Corp, Westbury, NY). The low-density (LD) cells were harvested, washed, and resuspended in the carrier. These LD cells were then used in the progenitor cell assays and the PBPC transplantation.

Growth Factors

rrSCF-PEG,8 rhG-CSF,9 rrSCF,10 recombinant human interleukin-6 (rhlL-6),11 and recombinant human erythropoietin (rhEpo)12 were obtained from Amgen Inc (Thousand Oaks, CA). rrSCF-PEG has been shown to be required for activity in vivo studies with rodents because the unpegylated form has little, if any, in vivo activity (McNiece IK and Zsebo KM, unpublished data). Recombinant murine IL-3 (rmIL-3)13 was obtained from PeproTech Inc (Rocky Hill, NJ). All factors were purified to greater than 99% purity.

Progenitor Cell Assays

GM-CFC and HPP-CFC. Both 2.5 × 10⁴ and 1.0 × 10⁵ LD cells were cultured in a double-layer agar-based assay system in triplicate, as previously described.14 Each culture contained optimal doses of rrSCF (100 ng/culture), rmIL-3 (2.5 ng/culture), and absolute numbers of circulating cells with in vivo repopulating potential.
GM-CFC- and HPP-CFC-derived colonies were scored in situ, as the transplant potential of the mobilized PBPC. Maximal transplants of 200,000 cells were increased with a plateau occurring at doses of 100 pg/kg of rrSCF-PEG for 7 days in mice treated with 200 pg/kg/d of rrSCF-PEG compared with approximately 2.0 \times 10^7 WBC/mL of peripheral blood of rrSCF-PEG-treated mice as compared with untreated mice.

To further evaluate the potential of high doses of rrSCF-PEG to mobilize PBPC, mice were treated with 200 pg/kg/d, rhG-CSF alone (200 pg/kg/d), and the combination of the two cytokines are shown in Fig 1. No significant increase in WBC numbers was obtained with rrSCF-PEG treatment compared with untreated mice, whereas rhG-CSF stimulated more than twofold higher WBC numbers (Table 1 and Fig 1). The combination of rrSCF-PEG and rhG-CSF stimulated a synergistic increase in WBC numbers (Fig 1). No significant differences were obtained for bone marrow WBC numbers (Fig 1).

In mice treated with both rrSCF-PEG and rhG-CSF, an approximately threefold increase per 100,000 LD cells in mature progenitor cells (GM-CFC) resulted compared with rhG-CSF alone (Fig 2). Only a slight increase in primitive progenitor cells (HPP-CFC) was obtained in this setting (Fig 2). However, when corrected for the WBC number increases in the bone marrow of these mice. Transplantation of 1.0 \times 10^7, 5.0 \times 10^6, or 2.5 \times 10^6 LD cells from untreated animals resulted in 100%, 20%, and 10% survival of irradiated mice, respectively, whereas 2.5 \times 10^5, 5.0 \times 10^5, or 1.0 \times 10^6 LD cells from rrSCF-PEG treated mice all resulted in 100% survival. This represents a 40-fold higher number of cells capable of rescuing lethally irradiated mice in the peripheral blood of rrSCF-PEG–treated mice as compared with untreated mice.

Based on the ability of low doses of rhSCF to synergize with other hematopoietic cytokines in vitro, including rhG-CSF, we examined the potential of low doses of rrSCF-PEG to synergize with rhG-CSF in PBPC mobilization. A dose response was performed with rhG-CSF alone. Plateau numbers of WBC were obtained with a dose of 100 pg/kg/d, and subsequent experiments were performed with a dose of 200 pg/kg/d of rhG-CSF for maximal mobilization. The WBC numbers from mice treated with rrSCF-PEG alone (25 pg/kg/d), rhG-CSF alone (200 pg/kg/d), and the combination of the two cytokines are shown in Table 1. A total of 1.0 \times 10^7 LD cells were cultured in a methylcellulose-based assay system in quadruplicate, as previously described. Each culture contained optimal doses of rrSCF, rIL-3, and rhEpo (4 U/culture) and was incubated for 14 days, after which BFU-E-derived colonies were scored in situ by hemoglobinization content.

PBPC Transplantation

Twelve-week-old female (C57BL/6J \times DBA/2J) F, mice (Charles River Laboratories) were irradiated at 1,150 rad using a cesium source, which was administered as a split-dose of equal intensity 4 hours apart. Mice were injected intravenously with the carrier or various numbers of LD cells 4 hours after the second irradiation. All experiments contained irradiation controls, in which 10 mice were irradiated without LD cell transplantation to show the lethality of the irradiation dose.

RESULTS

A dose-related increase in peripheral blood WBC numbers resulted with a daily injection of rrSCF-PEG for 7 days (Table 1). Approximately 9.0 \times 10^7 WBC/mL of peripheral blood were obtained in mice treated with 200 pg/kg of rrSCF-PEG compared with approximately 2.0 \times 10^7 in mice receiving only the carrier (Table 1). Both primitive (HPP-CFC) and mature (GM-CFC) progenitor cell numbers were increased with a plateau occurring at doses of 100 and 200 pg/kg (Table 1). A total of 2.0 \times 10^7 LD cells were transplanted into lethally irradiated recipients to determine the transplant potential of the mobilized PBPC. Maximal survival was obtained with cells from mice treated with 200 pg/kg with a dose-related decrease in survival, as shown in Table 1.
obtained with the combination rrSCF-PEG and rhG-CSF over rhG-CSF alone, significantly higher absolute numbers of HPP-CFC were released by the combination (Table 2). Similarly, whereas only a slight increase in the incidence of erythroid progenitor cells (BFU-E) was obtained with rrSCF-PEG and rhG-CSF as compared with rhG-CSF alone, the absolute numbers of BFU-E were greatly increased (data not shown). Transplantation of the mobilized PBPC from rrSCF-PEG plus rhG-CSF–treated animals resulted in equivalent or greater survival of lethally irradiated mice compared with animals receiving transplants of rhG-CSF–mobilized PBPC (Table 3). When corrected for the increased WBC count, the combination of rrSCF-PEG plus rhG-CSF resulted in an absolute increase in the number of cells capable of rescuing lethally irradiated mice compared with rhG-CSF alone.

**Table 2. Absolute Number of GM-CFC and HPP-CFC per Milliliter in Mobilized Peripheral Blood**

<table>
<thead>
<tr>
<th>Factor Treatment</th>
<th>GM-CFC</th>
<th>HPP-CFC</th>
</tr>
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<tbody>
<tr>
<td>rrSCF-PEG (25 μg/kg/d)</td>
<td>13,311</td>
<td>3,081</td>
</tr>
<tr>
<td>rhG-CSF (200 μg/kg/d)</td>
<td>36,108</td>
<td>10,492</td>
</tr>
<tr>
<td>rrSCF-PEG + rhG-CSF</td>
<td>178,408</td>
<td>22,532</td>
</tr>
</tbody>
</table>

* Splenectomized mice (n = 10) were treated with the growth factors shown for 7 days, at which time peripheral blood was harvested and LD cells were fractionated and cultured as described in the Materials and Methods.

† Absolute GM-CFC and HPP-CFC numbers were calculated by multiplying the mean colony numbers per 1.0 × 10^6 cells (from Fig 2) by the WBC numbers (from Table 1) and dividing by 1.0 × 10^6. This represents an approximation of total GM-CFC and total HPP-CFC per milliliter peripheral blood after factor treatment.

**Discussion**

The use of G-CSF for PBPC mobilization for support of high-dose chemotherapy has enabled more rapid engraftment of both neutrophils and platelets. However, a significant period of neutropenia and thrombocytopenia still occurs with PBPC transplantation. Other means of mobilization, including chemotherapy and/or growth factors such as GM-CSF or IL-3, result in nonoptimal mobilization. Current protocols require at least three aphereses to collect enough cells for transplantation. In a number of patients, insufficient numbers of CD34+ and/or GM-CFC in the harvest make them ineligible for transplantation. Mobilization techniques that would give increased numbers of progenitor cells would potentially improve PBPC transplantation and allow more rapid engraftment. The in vitro and in vivo properties of SCF have suggested exciting potentials for clinical use of this cytokine in a number of settings. In vitro low doses of SCF can synergize with other cytokines such as G-CSF and Epo to stimulate both primitive and mature progenitor cells. Both G-CSF and Epo have very little, if any, dose-limiting side effects. As shown in this study and in previous work by Andrews et al., treatment of mice or baboons with SCF results in increased numbers of peripheral WBC, primitive and mature progenitor cells, and cells with short term repopulating potential. Comparison of the effects of rrSCF-PEG (200 μg/kg/d) with rhG-CSF (200 μg/kg/d) in mice shows that rrSCF-PEG stimulates up to two-fold more circulating WBC consisting almost entirely of neutrophils. The quality of the mobilized PBPC as evaluated by the potential to rescue lethally irradiated mice suggests that SCF–mobilized cells are a better source of cells for transplantation than G-CSF–mobilized cells. We propose that G-CSF alone stimulates a shifting of mature neutrophils and mature progenitor cells from the bone marrow to...
the periphery, whereas SCF stimulates expansion of both primitive and mature progenitor cells in the bone marrow and a subsequent shift to the periphery.

Further data presented in this study show the potential of the combination of SCF plus G-CSF to mobilize PBPC. The combination of low doses of rrSCF-PEG (25 μg/kg) plus rhG-CSF (200 μg/kg) results in a synergistic increase in circulating WBC numbers, GM-CFC, BFU-E, HPP-CFC, and cells capable of rescuing lethally irradiated mice, as compared with mobilization with rhG-CSF alone. The low dose of rrSCF-PEG has little, if any, effect alone. The effective dose of rhG-CSF in clinical treatment is at least 1 log lower in humans than the optimal dose in mice. If an equivalent ratio occurs with SCF, the range of 2.5 to 5.0 μg/kg may be an effective dose in combination with G-CSF. In early clinical studies, SCF has been reported to be well tolerated at 10 μg/kg, suggesting that a dose of 2.5 to 5 μg/kg will be well tolerated by patients. The data presented in this study suggest that the combination will be effective in mobilizing PBPC.

In summary, this study presents data supportive of a role of low doses of SCF in combination with G-CSF for enhanced mobilization of PBPC. The data suggest that clinical trials with this combination may result in fewer apheresis harvests and, possibly, better engraftment.

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REFERENCES

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RA Briddell, CA Hartley, KA Smith and IK McNiece