The Effect of Stem Cell Factor on Irradiated Human Bone Marrow

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Abstract

This study evaluates the effect of recombinant human stem cell factor (SCF) on the in vitro response of human bone marrow progenitor cells to irradiation. Light density nonadherent mononuclear cells were isolated from human bone marrow and resuspended in either semisolid culture or liquid culture with or without 100 ng/ml SCF. After 24 h in culture, cells were irradiated and assessed for survival of erythroid burst-forming unit, granulocyte colony-forming unit(s), or granulocyte-macrophage colony-forming unit precursors in the presence of erythropoietin, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor, respectively. Incubation with SCF prior to irradiation (0-300 cGy) resulted in an increase in both absolute colony number and surviving fraction for erythroid burst-forming units, granulocyte colony-forming units, and granulocyte-macrophage colony-forming units as compared to cultures that did not contain SCF. The mean surviving fraction enhancement ratio after 100 cGy ranged from 1.2 to 3.7. An increased fraction of CD34+ progenitors in S-phase after exposure to SCF may explain in part the apparent radioprotective effect of SCF on human bone marrow progenitor cells.

Introduction

Blood count suppression substantially limits nonsurgical forms of cancer therapy and is a major source of morbidity and mortality. Hospitalizations for fever and neutropenia, life-threatening infectious episodes, and RBC and platelet transfusion requirements are the most frequently recognized problems associated with prolonged myelosuppression after aggressive chemotherapy and bone marrow transplantation regimens. Hematopoietic intolerance also inhibits the effective use of large field irradiation, the use of radiolabeled monoclonal antibodies, and the development of combined chemotherapy and irradiation regimens which have shown promise in malignant lymphomas and a variety of solid tumors. An agent which reduces the degree or duration of radiation therapy related myelosuppression may improve the therapeutic index for these treatments.

SCF (1) is a glycoprotein that acts on primitive multilineage blood cell progenitors in the marrow. In vitro, SCF alone has little colony-stimulating activity for human bone marrow cells, but it increases the number of colonies in response to erythropoietin, G-CSF and GM-CSF (2). In vivo, recombinant rat SCF treatment has been reported to stimulate hematopoietic recovery resulting in the survival of mice exposed to otherwise lethal doses of total body irradiation (3).

Materials and Methods

Growth Factors. Recombinant human SCF, G-CSF, GM-CSF, and erythropoietin were prepared and provided by Amgen (Thousand Oaks, CA). All growth factors were diluted in phosphate buffered saline containing 0.1% human serum albumin. SCF, G-CSF, and GM-CSF were used at a final concentration of 100 ng/ml unless otherwise specified. Erythropoietin was used at a final concentration of 2 units/ml.

Human Bone Marrow Cells. Bone marrow samples from both normal donors and cancer patients were generously provided by the Stanford University Hospital Bone Marrow Transplant Laboratory. Results were similar with both groups of donors. All bone marrow donors signed an informed consent form prior to the procedure in accordance with the Stanford Human Subjects Committee. Fresh marrow was used for all experiments immediately following the harvest procedure. Light-density (<1.077 g/cc) nonadherent marrow cells were isolated by placing buoyant mononuclear cells from Ficoll-Hypaque centrifugation on plastic tissue culture dishes in IMDM supplemented with 20% FCS. Nonadherent cells were recovered after overnight incubation at 37°C in 5% CO2, as previously described (5).

Colony-forming Assays. Isolated marrow cells were resuspended in either semisolid culture or liquid culture with and without SCF. Semisolid cultures were prepared in 24-well tissue culture plates containing IMDM supplemented with 20% FCS. 1.1% methylcellulose, 1% deionized bovine serum albumin, 5 × 10⁻³ M 2-mercaptoethanol, and the appropriate growth factor. After 24 h, the cultures were irradiated with 100 to 200 cGy 250 KVP x-rays. The irradiated cultures as well as unirradiated control cultures were assessed for survival of BFU-E, CFU-G, or CFU-GM precursors in the presence of erythropoietin, G-CSF, or GM-CSF, respectively.

Liquid cultures were prepared in 6-well tissue culture plates containing IMDM and 20% FCS, with and without SCF. After 24 h, the liquid cultures were irradiated with 100 cGy using a 137-Cesium γ source immediately after irradiation the cells were washed and plated in semisolid culture without SCF but with erythropoietin, G-CSF, or GM-CSF to assess survival of BFU-E, CFU-G, and CFU-GM, respectively. Colonies (≥50 cells) were counted after 10 days of incubation at 37°C in a humidified incubator containing 5% CO2.

Results are expressed as mean colony counts ± SE and are based upon counts from 3 to 6 wells for each experimental group. The surviving fraction for each precursor was calculated by dividing the mean colony count at each radiation dose by the mean colony count of the unirradiated control group. The surviving fraction enhancement ratio was calculated by dividing the surviving fraction for the SCF-treated cells by the surviving fraction for the untreated cells for each radiation dose. D37 values were determined using the FIT 2.03 program (6). All experiments were repeated three times and produced similar results.

Progenitor S-Phase Fraction Determination. CD34+ cells were isolated using the Microcollector Stem Cell Kit (Applied Immune Sciences, Menlo Park, CA). Briefly, light density mononuclear bone marrow cells were enriched for progenitors by incubation for 60 min in a T-150 culture flask with covalently bound soybean agglutinin. The supernatant was harvested and incubated for 60 min in a T-25 culture flask containing covalently bound CD34 monoclonal antibodies. The isolated CD34+ cells were then collected from the flask and incubated in IMDM and 20% FCS at 37°C with 5% CO2, with and without SCF, for 24 or 48 h prior to fixation in 70% ethanol and subsequent staining with propidium iodide. The S-phase fraction of the propidium iodide

Received 6/3/93; accepted 7/19/93.

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1 This work was supported in part by fellowships from the American Society for Therapeutic Radiology and Oncology Research and Education Development Fund and the Radiological Society of North America Research and Education Fund, an American Cancer Society Clinical Oncology Career Development Award, and a grant from Amgen, Inc.

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3 The abbreviations used are: SCF, stem cell factor; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; BFU-E, erythroid burst-forming unit(s); CFU-G, granulocyte colony-forming unit(s); CFU-GM, granulocyte-macrophage colony-forming unit(s); PCS, fetal calf serum; IMDM, Iscove’s Modified Dulbecco’s Medium.
stained cells was then determined by measuring DNA content with a fluorescence-activated cell sorter. The mean of five samples from each experimental group is reported.

**Statistics.** Statistical comparisons between treatment groups were made with the paired one-tailed Student's t test.

**Results**

**Semisolid Culture Experiments.** The addition of SCF to the semisolid culture medium enhanced the survival of colony forming units from human bone marrow. In pilot studies, the addition of SCF (100 ng/ml) 24 h prior to irradiation was found to result in maximal enhancement of colony formation (data not shown). The increase in mean number of surviving progenitor cells ranged from 1.5- to over 3-fold and was present at all radiation doses for each of the three progenitor lines studied (Fig. 1).

The percentage increase in surviving progenitors with the addition of SCF was larger for the irradiated cells when compared to the unirradiated controls. This is illustrated by an improvement in surviving fraction with the addition of SCF for nearly all experimental conditions (Table 1). BFU-E and CFU-G demonstrated the largest SCF associated increase in surviving fraction with a mean improvement of 3.8- and 2.5-fold, respectively. For CFU-GM, although significant increases in absolute colony numbers were clearly demonstrated (Fig. 1), only marginal improvements in surviving fraction were observed. The mean surviving fraction enhancement ratio for all semisolid experiments combined was 2.4.

D$_{37}$ was calculated for each progenitor line using the surviving fraction data from the semisolid experiments. D$_{37}$ values with and without SCF were 141 and 100 cGy, 151 and 104 cGy, and 119 and 103 cGy for BFU-E, CFU-G, and CFU-GM, respectively.

**Liquid Culture Experiments.** In order to eliminate the known synergistic interaction of SCF with recombinant human colony stimulating factors, marrow cells were incubated in liquid culture with and without SCF, irradiated with 100 cGy, and then washed prior to plating in semisolid medium with the appropriate growth factor to assess for survival of BFU-E (A), CFU-G (B), and CFU-GM (C). Results represent the mean of 3 or 4 samples for each data point ± SEM. Two additional experiments gave similar results.

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>XRT (cGy)</th>
<th>SCF mean</th>
<th>Control mean</th>
<th>Enhancement ratio</th>
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<tr>
<td>EPO</td>
<td>100</td>
<td>0.56 (0.11)</td>
<td>0.32 (0.10)</td>
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<td>EPO</td>
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<td>8.0$^*$</td>
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<td>G-CSF</td>
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<td>0.39 (0.06)</td>
<td>1.7 (0.3)</td>
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<td>0.09 (0.04)</td>
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<td>0.02 (0.00)</td>
<td>3.7 (1.4)</td>
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<tr>
<td>GM-CSF</td>
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<td>0.16 (0.02)</td>
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<tr>
<td>GM-CSF</td>
<td>300</td>
<td>0.03 (0.01)</td>
<td>0.05 (0.02)</td>
<td>0.7 (0.3)</td>
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</tbody>
</table>

*$^*$ Indicates a statistically significant increase in surviving fraction for the SCF treated cells (P ≤ 0.05, paired one-tailed Student’s t test).

**Fig. 2.** Erythroid and myeloid progenitor cell survival after incubation in liquid culture with or without SCF for 24 h prior to irradiation. Cells were washed free of SCF after irradiation with 100 cGy and then plated in semisolid culture with the appropriate growth factor to assess for survival of BFU-E (A), CFU-G (B), and CFU-GM (C). Results represent the mean of 3 or 4 samples for each data point ± SEM. Two additional experiments gave similar results.

**Progenitor S-Phase Fraction.** CD34+ bone marrow cells were isolated and incubated in liquid culture with and without SCF for 24 or 48 h and then subjected to cell cycle analysis. The mean percentage of CD34+ cells in S-phase after 24 h was 14.1 ± 0.5 (mean ± SEM)
for the SCF-treated cells compared to 11.5 ± 1.2 for the untreated control cells ($P = 0.09$). After 48 h, the mean S-phase percentage was 15.5 ± 1.0 for the SCF-treated cells and 10.7 ± 0.5 for the control samples ($P = 0.01$).

**Discussion**

We have demonstrated that SCF enhances human bone marrow progenitor cell (BFU-E, CFU-G and CFU-GM) survival after *in vitro* irradiation. The optimal regime for maximal colony formation is incubation of cells with SCF (100 ng/ml) for 24 h prior to irradiation. Survival enhancement was observed for both erythroid and myeloid precursors after single radiation doses up to 300 cGy. SCF treatment resulted in an increase in the absolute number of surviving progenitors and in the surviving fraction for nearly all experimental conditions. The mean enhancement in surviving fraction was 2.4-fold for both the semisolid and liquid culture experiments.

SCF reportedly acts on CD34+ precursor cells and facilitates the proliferation response to other hematopoietic growth factors (7). This action is a direct one since neither CD34+ nor CD34- accessory cells are required for the proliferative response to SCF. We have demonstrated that treating CD34+ cells with SCF results in a larger fraction of cells in S-phase, which is the most radioresistant phase of the cell cycle (8, 9). This may explain in part the apparent radioprotective effect of SCF on human bone marrow progenitor cells.

The results reported here suggest that SCF may be effective in reducing the degree or duration of myelosuppression associated with clinical radiation therapy. Prior studies have shown that SCF protects mice (3) and dogs (4) from hematopoietic death after otherwise lethal doses of radiation therapy. Future experiments will be designed to further elucidate the mechanism of action of SCF and to evaluate the potential *in vivo* radioprotective effects of SCF using severe combined immunodeficiency mice reconstituted with human bone marrow (10).

If *in vivo* activity for human marrow is confirmed by these experiments, phase I clinical trials of SCF for the prevention of treatment-related myelosuppression may be warranted. By reducing the degree or duration of myelosuppression associated with clinical radiation therapy, SCF may increase the therapeutic index of radiation therapy and allow higher doses of radiation to be delivered without increasing toxicity, which may ultimately increase local control and cure rates.

**Acknowledgments**

The authors thank Dr. Ian McNiece and Dr. Brian McGwire for their advice regarding experimental design and for the provision of growth factors. We also thank Dr. Karl Blume and Dr. Robert Negrin of the Stanford University Bone Marrow Transplant Laboratory for generously providing bone marrow samples. We gratefully acknowledge the technical assistance of Bill Sutherland, Sylvie Paroski, Nancy Ginzton, and Mary Kovacs.

**References**


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