Stem Cell Factor and Basic Fibroblast Growth Factor Are Synergistic in Augmenting Committed Myeloid Progenitor Cell Growth

By Janice L. Gabrilove, Kevin White, Zia Rahman, and E. Lynnette Wilson

Stem cell factor (SCF) and basic fibroblast growth factor (bFGF) are hematopoietic cytokines produced by bone marrow stromal cells. It is known that, although SCF and bFGF have limited clonogenic activity on their own, they can augment colony-stimulating factor (CSF)-mediated progenitor cell growth. Because these factors are both sequestered by stromal cells, we examined their interaction on progenitor cell growth in conjunction with granulocyte-macrophage–CSF (GM-CSF). In this study, we show that clonogenic growth derived from low-density bone marrow cells stimulated by GM-CSF is significantly augmented (P < .001) in the presence of maximal (100 ng/mL) concentrations of SCF in combination with 100 ng/mL of bFGF.

Stem cell factor (SCF) is a hematopoietic and tissue growth factor that serves as the ligand for the c-kit oncogene. SCF stimulates little colony growth when used alone, but has been shown to augment the in vitro proliferation of both myeloid and lymphoid hematopoietic progenitor cells. In the presence of erythropoietin, granulocyte colony-stimulating factor (G-CSF), interleukin-3 (IL-3), or granulocyte-macrophage–CSF (GM-CSF). It has been proposed that SCF produced locally at high concentrations by marrow stromal cells acts as an “anchor” factor and permits stem cells to respond to physiologic concentrations of other cytokines.

There is evidence that basic fibroblast growth factor (bFGF) can act as a hematopoietic cytokine. It is produced by a potenti and is a potent mitogen for hematopoietic stromal cells. It is found in megakaryocytes and cells of the granulocyte lineage in vivo, and it enhances megakaryopoesis in human long-term bone marrow cultures. In the presence of bFGF and insulin-like growth factor, single human bone marrow stem cells give rise to the hematopoietic microenvironment as well as hematopoietic progenitor cells. bFGF also augments the proliferation of progenitor cells when added in conjunction with other growth factors and it counteracts the suppressive effects of transforming growth factor-β1 (TGF-β1) on human myeloid progenitor cells. Recently, FGF receptor genes have been found to be expressed in normal human megakaryocytes and some leukemic cells.

bFGF and SCF are both stromal cell-derived factors. The combination of these two cytokines in conjunction with leukemia inhibitory factor has already been shown to stimulate the long-term proliferation of mouse primordial germ cells. Because of this synergy in a stem cell population, we investigated whether bFGF and SCF would be synergistic in augmenting GM-CSF–mediated human progenitor cell growth of hematopoietic precursor cells.

Materials and Methods

Reagents. Recombinant human SCF, recombinant human G-CSF, recombinant human IL-3, and recombinant human GM-CSF were obtained from Amgen (Thousand Oaks, CA). Human GM-CSF and G-CSF have a specific activity of 1 x 10^8 U/mg protein in a standard bone marrow colony-forming unit granulocyte-macrophage (CFU-GM) assay. Stock vials of human SCF were stored at -70°C, whereas stock vials of human G-CSF, IL-3, and GM-CSF were stored at 4°C. Recombinant human bFGF was obtained from Synergen (Boulder, CO). Stock vials were stored at -20°C. For each experiment, all factors were diluted in serum-containing medium on the day of use. A fluorescein isothiocyanate (FITC)-conjugated anti-CD34 antibody, HPCA-I, was purchased from Becton Dickinson (San Jose, CA). Human gamma globulin was obtained from Miles Inc (Cutter Biological, Elkhart, IN).

Cell separation techniques. Bone marrow cells were obtained from healthy volunteers after informed consent. The mononuclear cells were isolated by centrifugation on Ficoll-Hypaque gradients (1.077 g/mL; Pharmacia Fine Chemicals, Piscataway, NJ), washed twice in phosphate-buffered saline (PBS), and suspended in Iscove’s modified Dulbecco’s medium (IMDM) containing 10% fetal calf serum (FCS; Hyclone, Logan, UT) supplemented with penicillin (100 U/mL; GIBCO, Grand Island, NY), streptomycin (100 μg/mL; GIBCO), and 3 mg/mL glutamine (GIBCO). These cells were used as target cell populations for the CFU-GM progenitor cell assay.

CD34+ enriched cell populations were prepared as follows. Mononuclear cells were washed twice in PBS and resuspended at 15 x 10^6 cells/mL in PBS containing 0.1% human gamma globulin and incubated for 15 minutes at room temperature. Four-milliliter suspensions were then transferred to AIS MicroCELLector T-25 flasks coated with soybean agglutinin (Applied Immune Sciences, Menlo Park, CA) to remove soybean agglutinin-positive cells as described. After 1 hour of incubation on a nonvibrating surface, might interact in concert with other hematopoietic cytokines to regulate stem cell proliferation and differentiation in hematopoietic niches in the bone marrow.

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GM-CSF + SCF + bFGF at 15 three times to remove any residual nonadherent cells. The nonadherent cell population was then washed twice in PBS, resuspended at 10^6 cells/mL. Ten milliliters of PBS containing 10% FCS was then added to each flask and incubated for 15 minutes at room temperature. Cell suspensions were subsequently transferred to AIS MicroCELlector T-25 flasks coated with anti-CD34 antibodies (Applied Immune Sciences). Nonadherent cells were removed after one hour incubation at room temperature on a nonvibrating surface. Flasks were washed three times to remove any residual nonadherent cells. Ten milliliters of PBS containing 10% FCS was then added to each flask and adherent cells were removed by manual agitation of the flasks. The final surface, supernatant cells were removed. Flasks were then washed at 15 x 10^6 cells/mL in PBS containing 0.1% human gamma globulin and incubated for 15 minutes at room temperature. Cell suspensions were subsequently transferred to AIS MicroCELlector T-25 flasks coated with anti-CD34 antibodies (Applied Immune Sciences). Nonadherent cells were removed after one hour incubation at room temperature on a nonvibrating surface. Flasks were washed three times to remove any residual nonadherent cells. Ten milliliters of PBS containing 10% FCS was then added to each flask and adherent cells were removed by manual agitation of the flasks. The final cell population was found to contain 87% to 97% CD34+ cells, as analyzed by flow cytometry using an FITC-conjugated anti-CD34 antibody, HPCA-1.

CFU-GM. Low-density (1 x 10^5 cells/mL) or CD34+ (5 x 10^3 cells/mL) were cultured in 35-mm tissue culture dishes (Corning, Corning, NY) in McCoy's modified assay medium containing 0.3% agar (DIFCO, Detroit, MI) and 10% FCS. Cultures were stimulated by the addition of the following growth factor concentrations alone or in combination: 0.1 ng/mL, 1.0 ng/mL, 10 ng/mL, or 20 ng/mL of GM-CSF; 10 ng/mL, 100 ng/mL, or 1,000 ng/mL of bFGF; 100 ng/mL or 1,000 ng/mL of SCF; 100 ng/mL G-CSF; and 1.0 ng/mL, 10 ng/mL, or 100 ng/mL IL-3.

RESULTS

Low-density bone marrow cells were cultured in the presence of GM-CSF, SCF, or bFGF; GM-CSF + SCF; GM-CSF + bFGF; or GM-CSF + SCF + bFGF (Fig 1). No growth was observed in the presence of bFGF or SCF alone (Fig 1). The addition of either SCF (100 ng/mL) or bFGF (100 ng/mL) to cultures stimulated by GM-CSF (20 ng/mL) resulted in a twofold increase in day-7 and day-14 progenitor cell growth (Fig 1). The addition of both SCF (100 ng/mL) and bFGF (100 ng/mL) to GM-CSF-treated cultures resulted in a further increase in colony growth (1.7-fold) over that with GM-CSF + SCF or GM-CSF + FGF (Fig 1).

To determine whether the synergism observed with SCF + bFGF in combination with GM-CSF was direct or indirect, we examined the effect of these growth factors on the proliferation of CD34+ cells (Fig 2). No clonogenic growth was observed when CD34+ cells were stimulated by GM-CSF (20 ng/mL), a four-fold (day 7; P < .001) and 2.4-fold (day 14; P < .001) increase in the mean number of day-7 clusters and day-14 colonies was observed. When CD34+ cells were stimulated by GM-CSF (20 ng/mL), SCF (100 ng/mL), and bFGF (100 ng/mL) in combination, a sevenfold (day 7; P < .001) and 3.6-fold (day 14; P < .001) augmentation in progenitor cell growth was observed, demonstrating the potent synergism of these two growth factors in enhancing GM-CSF-supported progenitor cell growth. No synergistic augmentation of progenitor cell growth was observed when suboptimal concentra-

![Fig 1. Response of low-density bone marrow cells to bFGF, SCF, and GM-CSF alone and in combination. Data represent the mean ± standard deviation for eight plates scored on day 7 and day 14 in a representative experiment. For these studies, 1 X 10^6 low-density bone marrow cells were cultured for 7 and 14 days, respectively.*P < .001. 1bFGF, 100 ng/mL; SCF, 100 ng/mL; GM-CSF, 20 ng/mL.](image)

**FIG 1.** Response of low-density bone marrow cells to bFGF, SCF, and GM-CSF alone and in combination. Data represent the mean ± standard deviation for eight plates scored on day 7 and day 14 in a representative experiment. For these studies, 1 X 10^6 low-density bone marrow cells were cultured for 7 and 14 days, respectively.*P < .001. 1bFGF, 100 ng/mL; SCF, 100 ng/mL; GM-CSF, 20 ng/mL.

![Fig 2. Response of CD34+ progenitor cells to bFGF, SCF, and GM-CSF alone and in combination. The data represent the mean number of day-7 and day-14 clusters and colonies ± standard deviation of four separate experiments. In these experiments, eight plates seeded with 5 X 10^5 CD34+ cells were scored for colony and cluster growth on days 7 and 14 of culture. *P < .001. 1bFGF, 100 ng/mL; SCF, 100 ng/mL; GM-CSF, 20 ng/mL.](image)

**FIG 2.** Response of CD34+ progenitor cells to bFGF, SCF, and GM-CSF alone and in combination. The data represent the mean number of day-7 and day-14 clusters and colonies ± standard deviation of four separate experiments. In these experiments, eight plates seeded with 5 X 10^5 CD34+ cells were scored for colony and cluster growth on days 7 and 14 of culture. *P < .001. 1bFGF, 100 ng/mL; SCF, 100 ng/mL; GM-CSF, 20 ng/mL.
Table 1. Synergistic Augmentation of IL-3-Stimulated Progenitor Cell Growth by bFGF and SCF

<table>
<thead>
<tr>
<th>Factor (ng/mL)</th>
<th>Day 7 Clusters</th>
<th>Day 14 Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SCF (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>FGF (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 (1)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 (10)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 (100)</td>
<td>59 ± 9</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>IL-3 (1) + SCF (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 (1) + FGF (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 (1) + SCF (100) + FGF (100)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 (10) + SCF (100)</td>
<td>30 ± 3*</td>
<td>41 ± 6*</td>
</tr>
<tr>
<td>IL-3 (10) + FGF (100)</td>
<td>15 ± 4*</td>
<td>24 ± 3†</td>
</tr>
<tr>
<td>IL-3 (10) + SCF (100) + FGF (100)</td>
<td>67 ± 15*</td>
<td>71 ± 14†</td>
</tr>
<tr>
<td>IL-3 (100) + SCF (100)</td>
<td>63 ± 28*</td>
<td>82 ± 3†</td>
</tr>
<tr>
<td>IL-3 (100) + FGF (100)</td>
<td>60 ± 5†</td>
<td>82 ± 3†</td>
</tr>
<tr>
<td>IL-3 (100) + SCF (100) + FGF (100)</td>
<td>108 ± 19†</td>
<td>123 ± 6†</td>
</tr>
</tbody>
</table>

Values are 5 x 10^4 CD34+ cells/plate and are the mean ± standard deviation of four plates scored on either day 7 or day 14 of culture.
* P < .01.
† P < .001.
‡ P < .002.

DISCUSSION

In this study, we show that bFGF in combination with optimal concentrations of SCF is synergistic in significantly augmenting GM-CSF– and IL-3– but not G-CSF–mediated myeloid progenitor cell growth. In addition, our data show that this effect is even more pronounced when purified progenitors are used as targets. This latter observation suggests that the induction of other hematopoietic growth factors by accessory cells by bFGF and/or SCF is not likely to be responsible for the enhanced clonogenic growth of CFU-GM.

It is known that optimal proliferation of both early and committed human and murine progenitors requires stimulation by multiple hematopoietic growth factors. SCF, which has little capacity to support progenitor cell growth alone, has been shown to stimulate significantly the growth of hematopoietic colonies. SCF has also been shown to restore the proliferative response of both unfractionated bone marrow, partially and highly purified SCA1−Lin− cells to subliminal concentrations of macrophage-CSF, G-CSF, GM-CSF, IL-1, and IL-3. This has led Lowry et al. to propose a model of control of the hematopoietic microenvironment in which SCF at locally high concentrations on the stromal cell surface enables the stem cells to respond to low physiologic concentrations of other cytokines. It is possible that bFGF might have a similar permissive effect to that noted for SCF.

bFGF is detectable in human megakaryocytes, platelets, and myeloid cells in vivo and is produced by stromal cells in vitro. In vitro, in human bone marrow cultures, bFGF binds to a cell surface phosphorylaminositol-linked heparan sulfate proteoglycan. Heparan sulfate proteoglycans also mediate the attachment of progenitor cells to stroma. Thus, it is possible that, within the stromal microenvironment, bFGF might be locally sequestered by heparan sulfate proteoglycans and that bFGF, in conjunction with SCF and other cytokines, might interact with and regulate the proliferation of adjacent hematopoietic stem cells.

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