Recombinant Human Stem Cell Factor Enhances the Formation of Colonies by CD34⁺ and CD34⁺lin⁻ Cells, and the Generation of Colony-Forming Cell Progeny From CD34⁺lin⁻ Cells Cultured With Interleukin-3, Granulocyte Colony-Stimulating Factor, or Granulocyte-Macrophage Colony-Stimulating Factor

By Irwin D. Bernstein, Robert G. Andrews, and Krisztina Zsebo

We tested the ability of recombinant human stem cell factor (SCF) to stimulate isolated marrow precursor cells to form colonies in semisolid media and to generate colony-forming cells (CFC) in liquid culture. SCF, in combination with interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), or granulocyte colony-stimulating factor (G-CSF) caused CD34⁺ cells to form increased numbers of granulocyte-macrophage colonies (CFU-GM), and to form macroscopic erythroid burst-forming units (BFU-E) in the presence of IL-3, erythropoietin (Epo), and SCF. We tested isolated CD34⁺lin⁻ cells, a minor subset of CD34⁺ cells that did not display antigens associated with lymphoid or myeloid lineages, and CD34⁺lin⁻ cells, which contain the vast majority of CFC, and found that the enhanced colony growth was most dramatic within the CD34⁺lin⁻ population. CD34⁺lin⁻ cells cultured in liquid medium containing SCF combined with IL-3, GM-CSF, or G-CSF gave rise to increased numbers of CFC. Maximal numbers of CFU-GM were generated from CD34⁺lin⁻ cells after 7 to 21 days of culture, and required the presence of SCF from the initiation of liquid culture. The addition of SCF to IL-3 and/or G-CSF in cultures of single CD34⁺lin⁻ cells resulted in increased numbers of CFC due to the proliferation of otherwise quiescent precursors and an increase in the numbers of CFC generated from individual precursors. These studies demonstrate the potent synergistic interaction between SCF and other hematopoietic growth factors on a highly immature population of CD34⁺lin⁻ progenitor cells.

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Rapid Communication

A hemopoietic factor produced by marrow stromal cells has recently been identified and determined to be the ligand for c-kit, a cell surface receptor with tyrosine kinase activity. This factor, termed stem cell factor (SCF), c-kit ligand, or mast cell growth factor, is the product of the SL locus in mice. In the mouse, recombinant rat SCF has been used to induce colony formation by marrow cells. When primitive precursors were obtained from post-5-FU treated mice, or were selected based on their lack of expression of antigens associated with mature myeloid or lymphoid cells, SCF acted in a synergistic manner with other growth factors, including interleukin-6 (IL-6), IL-3, and IL-1β, to induce colony formation. In humans, SCF also acts synergistically with IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and erythropoietin (Epo) to induce colony formation by unseparated human marrow cells.

In the present study, we determined the influence of recombinant human SCF alone and in combination with other hematopoietic growth factors on the formation of colonies by human hematopoietic progenitor cells that were isolated based on their expression of the CD34 antigen. We also examined the ability of SCF to stimulate generation of colony-forming cell (CFC) progeny from an immature subset of those cells that did not display antigens associated with the vast majority of committed myeloid progenitors and lymphoid cells.

Materials and Methods

Separation of marrow cells. Marrow samples were obtained after informed consent, and cells were separated by density gradient centrifugation. The cells were then washed, and stained using two-color indirect immunofluorescent antibody binding as described.

Cells were labeled simultaneously with IgG antibodies, 35.1 (CD2), 24.1 (CD10), HD37 (CD19), IF5 (CD20), p67 (CD33), and 7B9 stained with anti-μ chain specific goat antiserum labeled directly visualized in each well. CD34⁺ cells with low or negative staining with IgG antibodies (lin⁻) had fluorescence less than the upper 10% of cells stained with the control antibody, and the positively stained cells (lin⁺) were separated by five channels. In single cell experiments, one and two, CD34⁺ cells were gated to select only blast-sized cells.

CFC assays. Isolated cells were cultured in Iscove’s Modified Dulbecco’s Medium (IMDM), 0.9% methylcellulose (Terry Fox Cancer Center, Vancouver, BC, Canada) supplemented with 20% charge payment.

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fetal bovine serum (FBS) (HyClone, Logan, UT), 1% bovine serum albumin (BSA) (Intergen Co, Purchase, NY), $1 \times 10^{-4}$ mol/L 2-mercaptoethanol (Biorad Laboratories, Richmond, CA), 20% human placental conditioned medium (HPCM), IL-3 (100 ng/mL), and Epo (2 U/mL) as previously described, unless otherwise specified. Cultures were incubated at 37°C in 5% CO$_2$ in air incubators.

**Liquid cultures.** Cells were cultured in microtiter wells in IMDM-20% FBS supplemented with recombinant human growth factors. Cultures were fed twice weekly with appropriate media. At designated times the number of cells in each well was enumerated, and the cells were then harvested and plated for colony formation as described previously.

**Growth factors.** Recombinant human SCF, IL-3, G-CSF, GM-CSF, IL-6, and Epo were produced in Escherichia coli (supplied by Amgen, Thousand Oaks, CA). They were used as purified material at 100 ng/mL unless otherwise specified.

**RESULTS**

**Direct colony formation by CD34$^+$ subpopulations stimulated by SCF.** We isolated CD34$^+$ cells and subsets of these cells that were stained with the mixture of antibodies p67, 7B9 HD37, 1F5, 35.1, and 24.1 (lin$^+$) or those that were not (lin$^-$). In two experiments, the CD34$^+$lin$^+$ cells represented 91% and 91.2% of CD34$^+$ cells and the CD34$^+$lin$^-$ cells represented 7.3% and 7.1% of the CD34$^+$ cells. Based on the proportion of cells that were sorted into each population, the CD34$^+$lin$^-$ populations were determined to contain the vast majority (96% and 98%) of the total number of sorted CD34$^+$ CFC. Isolated CD34$^+$, CD34$^+$lin$^+$, and CD34$^+$lin$^-$ cells were also cultured with SCF alone or in combination with IL-3, Epo, GM-CSF, or G-CSF to determine the number and type of colonies formed.

The formation of colonies by isolated CD34$^+$ cells in response to individual factors was enhanced by the addition of SCF to those cultures with up to a sixfold increase in the number of CFU-GM (IL-3 + SCF; Table 1, experiment 2). In a third experiment in which the size of BFU-E was determined, 57% of the colonies in the CD34$^+$ population, 90% of the BFU-E in the CD34$^+$lin$^+$ population, and 65% of the BFU-E in the CD34$^+$lin$^-$ population were macroscopic in size ($>0.25$mm) in cultures with IL-3, Epo, and SCF, while no macroscopic colonies were seen in cultures containing only IL-3 and Epo (data not shown).

SCF had the greatest effect on the numbers of colonies formed by CD34$^+$lin$^-$ cells (Table 1). Only a few colonies were formed by isolated CD34$^+$lin$^-$ cells cultured in the presence of either SCF alone or in each of the other growth factors tested. In contrast, substantial increases in the number of colonies formed from CD34$^+$lin$^-$ cells were observed when SCF was added to other growth factors, with as high as an 18-fold increase in CFU-GM seen when GM-CSF was combined with SCF in experiment 1.

<p>| Table 1. Effects of SCF on Direct Colony Formation by CD34$^+$ Marrow Cells (colonies/10$^5$ cells) |</p>
<table>
<thead>
<tr>
<th>Factor</th>
<th>SCF</th>
<th>CD34$^+$</th>
<th>CD34$^+$lin$^+$</th>
<th>CD34$^+$lin$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>+</td>
<td>5 ± 1</td>
<td>3 ± 1</td>
<td>0</td>
</tr>
<tr>
<td>IL-3</td>
<td>+</td>
<td>69 ± 11*</td>
<td>23 ± 4*</td>
<td>50 ± 6*</td>
</tr>
<tr>
<td>Epo</td>
<td>+</td>
<td>7 ± 2</td>
<td>73 ± 7</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>IL-3 + Epo</td>
<td>+</td>
<td>54 ± 6*</td>
<td>96 ± 8</td>
<td>33 ± 3*</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>+</td>
<td>66 ± 11*</td>
<td>15 ± 2*</td>
<td>67 ± 13*</td>
</tr>
<tr>
<td>G-CSF</td>
<td>+</td>
<td>67 ± 10*</td>
<td>0</td>
<td>23 ± 9</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>+</td>
<td>7 ± 1</td>
<td>0</td>
<td>10 ± 10</td>
</tr>
<tr>
<td>IL-3</td>
<td>+</td>
<td>70 ± 6*</td>
<td>1 ± 1</td>
<td>30 ± 17</td>
</tr>
<tr>
<td>Epo</td>
<td>+</td>
<td>23 ± 5*</td>
<td>10 ± 1</td>
<td>17 ± 3*</td>
</tr>
<tr>
<td>IL-3 + Epo</td>
<td>+</td>
<td>54 ± 4</td>
<td>27 ± 3</td>
<td>100 ± 20</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>+</td>
<td>65 ± 8*</td>
<td>1 ± 1</td>
<td>43 ± 9</td>
</tr>
<tr>
<td>G-CSF</td>
<td>+</td>
<td>76 ± 6*</td>
<td>0</td>
<td>23 ± 15</td>
</tr>
</tbody>
</table>

Colony formation in semisolid media by isolated CD34$^+$, CD34$^+$lin$^+$ (CD2$^+$, CD10$^+$, CD19$^+$, CD20$^+$, CD33$^+$, 7B9$^+$) and CD34$^+$lin$^-$ (CD2$^-$, CD10$^-$, CD19$^-$, CD20$^-$, CD33$^-$, 7B9$^-$) cells. Data are mean ± SEM for triplicate cultures in each experiment. No macroscopic colonies were observed in cultures without SCF.

*Results different from cells cultured in the same factor without SCF, $P < .05$, two-sided Student’s $t$-test.
experiment 2, CFU-GM were not detected in cultures containing IL-3 or GM-CSF, but substantial numbers of CFU-GM were detectable when SCF was added to these cultures. Similarly, in experiment 1, BFU-E appeared only when SCF was added to IL-3, GM-CSF, or Epo. These BFU-E were mainly macroscopic in size, and no macroscopic colonies were observed in cultures not containing SCF.

The number of colonies formed by CD34+lin− cells was also enhanced by the addition of SCF. In experiment 1, BFU-E, including macroscopic BFU-E, were increased in cultures to which Epo, IL-3, or GM-CSF was added along with SCF, and at most up to a fivefold increase in CFU-GM and BFU-E was observed. No macroscopic BFU-E were seen in cultures to which SCF was not added. In general, colony formation by CD34+lin− cells was enhanced less than that seen using unfractionated CD34+ cells.

Influence of SCF on generation of CFC from CD34+lin− precursor cells in liquid culture. The ability of SCF to stimulate the generation of CFC from CD34+lin− and CD34+lin+ cells in liquid culture was assessed. After 7 days in liquid culture containing SCF or other individual growth factors alone, relatively small numbers of CFC were detected in cultures of either the lin− or the lin+CD34+ cells (Table 2). The addition of SCF to the other growth factors did not stimulate a significant increase in the numbers of CFC yielded by the CD34+lin− cells except for an increase of borderline significance (P < .1) in the case of IL-6, and in one of three additional experiments evaluating SCF and IL-3, a significant (4.5-fold) increase in CFC yield was caused by adding SCF to IL-3 (P < .05) (data not shown). In contrast, CD34+lin− cells cultured with SCF combined with other growth factors generated significantly more CFC that were primarily CFU-GM, but also included BFU-E. In this experiment, the addition of SCF to the combinations of IL-3 and G-CSF or IL-3 and IL-6 led to a significant increase in CFC yield (P < .05), while the addition of SCF to either IL-6, IL-3, or Epo led to an increase of borderline significance (P < .1). In two of the three additional experiments evaluating SCF with IL-3, we observed significant increases (17.1- and >24-fold) in CFC yield from CD34+lin− cells (P < .05) (data not shown).

The appearance of CFU-GM in cultures of CD34+lin− cells with SCF and IL-3 reached a peak at 7 to 21 days, while maximal numbers of BFU-E were found after 7 to 14 days. In contrast, CD34+lin− cells cultured with IL-3 led to a significant increase in CFC yield (P < .05), while the addition of SCF to either IL-6, IL-3, or Epo led to an increase of borderline significance (P < .1). In two of the three additional experiments evaluating SCF with IL-3, we observed significant increases (17.1- and >24-fold) in CFC yield from CD34+lin− cells (P < .05) (data not shown).

Seven-day cultures of CD34+lin− cells in IL-3 in which the addition of SCF to the cultures was delayed by 1, 2, 3, or 4 days demonstrated that delaying by 24 hours the addition of SCF to cultures containing IL-3 led to a decreased yield of CFU-GM by the CD34+lin− cells (Fig 2). In contrast, delay in the addition of SCF to CD34+lin− cells

<p>| Table 2. CD34+Lin− and CD34+Lin+ Cells: Generation of CFC After Seven Days in Liquid Culture (colonies/100 cells) |
|---|---|---|---|---|---|---|
| Factor* | SCF† | CD34+Lin− | CD34+Lin+ |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th>CFU-GM</th>
<th>BFU-E</th>
<th>CFU-GM</th>
<th>BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>+</td>
<td>14 ± 10</td>
<td>2 ± 2</td>
<td>12 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>IL-3</td>
<td>+</td>
<td>82 ± 26</td>
<td>0</td>
<td>10 ± 10</td>
<td>8 ± 8</td>
</tr>
<tr>
<td>Epo</td>
<td>+</td>
<td>26 ± 6</td>
<td>4 ± 0</td>
<td>4 ± 4</td>
<td>0</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>+</td>
<td>68 ± 21</td>
<td>12 ± 0</td>
<td>12 ± 8</td>
<td>0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>+</td>
<td>112 ± 16</td>
<td>6 ± 2</td>
<td>6 ± 6</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>IL-6</td>
<td>+</td>
<td>50 ± 26</td>
<td>14 ± 6</td>
<td>34 ± 14</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>IL-3 + G-CSF</td>
<td>+</td>
<td>62 ± 10</td>
<td>2 ± 2</td>
<td>10 ± 6</td>
<td>0</td>
</tr>
<tr>
<td>IL-3 + IL-6</td>
<td>+</td>
<td>78 ± 21</td>
<td>2 ± 2</td>
<td>14 ± 2</td>
<td>0</td>
</tr>
</tbody>
</table>

Cells were cultured at 25 cells per well in duplicate. Data are mean ± SEM per 100 cells initially placed in liquid culture, in a representative experiment. Cells were cultured for 7 days in liquid media containing the indicated factors, after which time the contents were removed and the cells were cultured in methylcellulose in the presence of GM-CSM, IL-3, and Epo to assess colony formation.

*IL-3, GM-CSF, G-CSF, IL-6, and SCF at 100 ng/mL and Epo at 2 U/mL.
†Results different from cells cultured in the same factor without SCF, P < .05, two-sided Student's t-test.

Fig 1. Generation over time of CFU-GM and BFU-E from CD34+lin− and CD34+lin+ marrow cells in liquid culture stimulated by SCF and IL-3. Isolated cells cultured at 25 cells per microtiter well were harvested at the time points indicated and cultured in semisolid media to assess colony growth (see Materials and Methods). Results are shown as the number of CFU-GM and BFU-E formed per 100 cells placed into liquid culture.
Figure 2. Effects on generation of CFU-GM in liquid cultures containing IL-3 by delaying the addition of SCF. Isolated cells cultured at 25 cells per microtiter well were harvested after 7 days and cultured in standard medium to assess colony growth (see Materials and Methods). Results are shown as the number of CFU-GM formed per 100 cells placed into liquid culture.

For up to 72 hours did not significantly affect the yield of CFU-GM after 7 days of liquid culture.

**SCF acts by inducing increasing numbers of CD34Lin- precursors to proliferate in the presence of additional growth factors.** To test if the increased yield of CFC in liquid cultures of CD34Lin- cells was due to increased proliferation of CFC or their generation from otherwise quiescent precursors, single CD34Lin- and CD34Lin+ cells were deposited into microtiter wells containing media supplemented with SCF, IL-3, or G-CSF alone or a combination of these growth factors. Additional aliquots of sorted cells were cultured directly in semisolid media and showed that for every 100 CD34Lin+ cells there were 6.5 ± 1.3, 12.8 ± 2.4, and 7.5 ± 0.6 CFU-GM and 14.3 ± 2.0, 28.4 ± 0.8, and 9.0 ± 1.5 BFU-E in experiments 1, 2, and 3, respectively. For every 100 CD34Lin+ cells there were 1.2 ± 0.6, 5.3 ± 1.2, and 4.7 ± 2.1 CFU-GM and 5.0 ± 0.8, 1.1 ± 0.6, and 3.0 ± 1.0 BFU-E in each experiment, respectively.

After 1 week, individual microtiter wells containing viable cells were harvested and the contents tested for CFC activity (Table 3). In experiments 1 and 2, 2.3% to 3.9% of wells initiated with a single CD34Lin- cell and cultured with SCF, IL-3, or G-CSF alone were found to contain one or more CFC, and in experiment 2, the combination of IL-3 and G-CSF caused 6.2% of single CD34Lin+ cells to generate CFC. When SCF was added to IL-3, 18.1% and 25.5% of CD34Lin- cells yielded CFC, and when it was added to IL-3 and G-CSF, 26% and 34% of all cells yielded CFC, in experiments 1 and 2, respectively. The high cloning efficiency of CD34Lin- cells in SCF, IL-3, and G-CSF was confirmed in the third experiment (25.5%). The addition of SCF to either IL-3 or IL-3 plus G-CSF also led to a significant increase in the average number of CFC yielded by a single precursor. Importantly, 40-, 41-, and 22-fold increases in the absolute number of CFC generated by the CD34Lin- cells were seen in liquid cultures containing SCF, IL-3, and G-CSF when compared with the number of CFC detected before liquid culture were found in experiments 1, 2, and 3, respectively. Furthermore, an occasional precursor cell yielded both CFU-GM and BFU-E when cultured with IL-3 and SCF or with IL-3, G-CSF, and SCF.

The yield from CFC by CD34Lin- cells was affected less by the addition of SCF to the cultures, with at most a 2.2- to 7.4-fold increase in CFC. In both experiments there was an apparent synergistic interaction between IL-3 and SCF based on the percentage of wells yielding CFC and the number of CFC per positive well. Of note, this percentage in experiment 2 for cultures containing IL-3 and SCF was not different from the percentage for cultures containing G-CSF alone. However, in both experiments the addition of G-CSF to the combination of IL-3 and SCF led to a marked decrease in the yield of CFU-GM.

**DISCUSSION**

The present study demonstrates the synergistic interaction between SCF and other known hematopoietic growth factors. SCF appears to act on CD34Lin- precursor cells to enable them to proliferate in response to other hematopoietic growth factors. This action is a direct one as neither CD34Lin+ or CD34Lin- accessory cells are required to mediate their activity. The enhanced growth of CFC by SCF is most prominently seen in a minor subset of the CD34Lin+ population that does not express a variety of antigens associated with lymphoid or myeloid lineages, including CD2, CD10, CD19, CD33, and 7B9. This CD34Lin+ population is depleted of the vast majority of cells present in the CD34Lin+ population, as well as most cells that form colonies in the presence of G-CSF, GM-CSF, or IL-3. However, when these factors are combined with SCF, CD34Lin- cells give rise to a remarkably increased number of colonies. Importantly, single cell analysis has demonstrated that SCF was essential for the proliferation of CD34Lin- cells otherwise incapable of forming colonies in the presence of other known hematopoietic growth factors; SCF also enhanced the generation of multiple CFC from single CD34Lin- cells cultured in the presence of one or more growth factors. Whether this reflected activation of less mature cells with a higher proliferative potential or more extensive proliferation by precursors with equivalent potential remains to be determined.

Thus, SCF dramatically affects a highly immature population of CD34Lin- cells when combined with certain other known hematopoietic growth factors. Studies in which the time of addition of SCF was varied suggest that SCF acts to maintain the viability of immature precursors and to render them competent to display a high proliferative potential in the presence of other hematopoietic growth factors.

Immature CD34Lin+ precursors depleted of most CFC by removal of CD33+ cells or HLA-DR+ cells have been shown to give rise to multiple CFC over a period of weeks to months when cultured over irradiated stroma.10,11,14,16 To what extent the CD34Lin- cells that respond to SCF in combination with other growth factors represent those cells
Table 3. Direct Effects of SCF on Generation of Colony-Forming Progeny From Single Precursor Cells in Culture

<table>
<thead>
<tr>
<th></th>
<th>Single CD34&lt;sup&gt;+&lt;/sup&gt; Lin&lt;sup&gt;-&lt;/sup&gt; Cell/Well</th>
<th>Single CD34&lt;sup&gt;+&lt;/sup&gt; Lin&lt;sup&gt;+&lt;/sup&gt; Cell/Well</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Wells With CFC</td>
<td>Average No. CFC/Pos Well</td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>E</td>
</tr>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-3</td>
<td>2.4</td>
<td>1.0</td>
</tr>
<tr>
<td>G-CSF</td>
<td>3.4</td>
<td>0.5</td>
</tr>
<tr>
<td>SCF</td>
<td>2.4</td>
<td>0.0</td>
</tr>
<tr>
<td>IL-3 + SCF</td>
<td>15.9</td>
<td>0.7</td>
</tr>
<tr>
<td>IL-3 + G-CSF + SCF</td>
<td>18.8</td>
<td>2.9</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-3</td>
<td>1.5</td>
<td>0.8</td>
</tr>
<tr>
<td>G-CSF</td>
<td>2.3</td>
<td>0.0</td>
</tr>
<tr>
<td>SCF</td>
<td>3.1</td>
<td>0.0</td>
</tr>
<tr>
<td>IL-3 + G-CSF</td>
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<td>0.0</td>
</tr>
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<td>IL-3 + SCF</td>
<td>21.6</td>
<td>2.3</td>
</tr>
<tr>
<td>IL-3 + G-CSF + SCF</td>
<td>32.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Experiment 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-3 + G-CSF + SCF</td>
<td>23.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Sorted single cells were cultured for 7 days in medium containing the indicated growth factor(s). Contents of individual wells, in which viable cells were detected visually, were cultured in methylcellulose with HPCM, IL-3, and Epo to assess colony formation. In experiment 1, 216 wells were cloned for lin<sup>-</sup> cells in IL-3, G-CSF, and SCF alone, 144 wells for lin<sup>-</sup> cultured with IL-3 and SCF, and 72 wells for lin<sup>-</sup> cells cultured in all three factors. For lin<sup>+</sup> cells, 72 wells were cloned for each group. In experiment 2, 144 wells were cloned for each group of lin<sup>+</sup> cells, except in all three factors in which 72 wells were done. Also in experiment 2, 72 wells were cloned for each group of lin<sup>-</sup> cells. As most wells with CD34<sup>+</sup><sup>-</sup> cells contained viable cells after 7 days of culture, only a portion of these cells were studied for CFC activity, and the results extrapolated. In experiment 3, 216 wells were cloned for each group of lin<sup>-</sup> and lin<sup>+</sup> cells. Results were corrected for efficiency of deposition of a single cell per well which was 96%, 90%, and 99%, in experiments 1, 2, and 3, respectively (see Materials and Methods).

* Different from values for IL-3, G-CSF, and SCF groups, P < .01.
† Different from values for IL-3, G-CSF, SCF, IL-3 + SCF groups, P < .01.
‡ Different from single positive wells.
§ Different from value for IL-3 + G-CSF, P < .01.
¶ Different from value for G-CSF, P < .01.

That give rise to CFC in long-term culture remains to be determined. However, the results in the present study suggest that SCF, when combined with other growth factors, may replace the function of stroma to induce the formation of CFC from their precursors, yielding a cloning efficiency of 25% or greater, an efficiency substantially greater than that observed with other hematopoietic growth factors. Furthermore, we have previously shown that stroma will support the formation of CFC from the larger-sized, presumably cycling CD34<sup>+</sup>CD33<sup>+</sup> cells over a period of weeks, while generation of CFC from smaller-sized, presumably quiescent, cells requires a longer time course. While we have observed that the SCF is active on larger-sized, presumably cycling cells (experiments 1 and 2, Table 3), whether SCF is similarly capable of supporting the growth of isolated noncycling cells remains to be determined.
Whether the use of serum-free medium will modify the influence of SCF and will enhance formation of greater numbers of multipotent progenitors also requires further study.

CD34+ cells, as well as marrow depleted of CD33+ cells, have been shown to be capable of establishing hematopoiesis in patients after myeloablative treatment. Although there is controversy regarding whether these cells are responsible for the long-term maintenance of hematopoiesis, the question of whether those CD34+lin- cells that respond to SCF include those cells capable of this in vivo hematopoietic activity is not known. In murine studies, a presumably comparable population of SCA-1+, Thy-1+, lin- cells was capable of establishing in vivo hematopoiesis in irradiated recipients.

The substantial ability of SCF to amplify the development of colony-forming cells from their presumably more immature CD34+lin- precursors may prove useful for inducing immature precursors to cycle for practical purposes. It may be possible to use SCF in vitro to expand the numbers of CFC for subsequent in vivo transplantation or in vivo to stimulate hematopoietic activity. Furthermore, on the assumption that SCF stimulates cells capable of in vivo reconstitution, it may be used to facilitate the proliferation that is apparently necessary for the integration of retroviral inserts in these cells for somatic cell gene therapy.

ACKNOWLEDGMENT

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REFERENCES


Recombinant human stem cell factor enhances the formation of colonies by CD34+ and CD34+lin- cells, and the generation of colony-forming cell progeny from CD34+lin- cells cultured with interleukin-3, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor

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