Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7420–7424, August 1991
Medical Sciences

Stem cell factor induces proliferation and differentiation of highly enriched murine hematopoietic cells

(stem cells/growth factors/hematopoiesis)

GIOVANNI MIGLIACCIO*†‡, ANNA RITA MIGLIACCIO*†, JAY VALINSKY*, KEITH LANGLEY§, KRISZTINA ZSEBO§, JAN W. M. VISSER¶, AND JOHN W. ADAMSON*

*New York Blood Center, New York, NY 10021; §Amgen, Thousand Oaks, CA 91320; †Istituto Superiore Sanità, Rome, Italy; and ¶Institute of Applied Radiobiology and Immunology, Netherlands Organization for Applied Scientific Research, Rijswijk, The Netherlands

Communicated by Eugene P. Cronkite, May 9, 1991

ABSTRACT **Recombinant rat stem cell factor (SCF) was studied for its ability to stimulate the growth of murine hematopoietic progenitor cells and to generate colony-forming cells (CFC) from highly enriched populations of hematopoietic cells. In serum-deprived cultures, SCF alone stimulated few colonies but interacted with a number of other hematopoietic growth factors, particularly interleukin 3, to promote colony formation. The most marked effect was on the generation of mixed-cell colonies. Hematopoietic cells were sorted into wheat-germ agglutinin-negative, monocyte-depleted, rhodamine 123 (Rh123)-bright or Rh123-dull cells. Historically, Rh123-bright cells are capable of short-term (<1 mo) marrow engraftment, whereas among Rh123-dull cells are cells capable of long-term marrow engraftment. Enriched cells (2.5×10^3) were placed into serum-deprived liquid cultures with various hematopoietic growth factors. Initially, the Rh123-bright and Rh123-dull cells had few CFC but, in the presence of interleukin 3 and SCF, Rh123-bright cells gave rise to >15,000 granulocyte/macrophage CFC, >1500 erythroid burst-forming cells, and >700 mixed-cell CFC by day 5. In contrast, Rh123-dull cells proliferated only in the presence of interleukin 3 and SCF, but total cell numbers rose to a peak of 18,000 by day 21, and one-third of the cells were CFC. Thus, SCF, in combination with other growth factors, can generate large numbers of CFC from pre-CFC and appears to act earlier than hematopoietic growth factors described to date.**

An important goal of experimental hematology is understanding the factors that control proliferation and differentiation of pluripotent hematopoietic stem cells (PSC). In pursuit of this goal, several methods have been described recently to purify PSC from rodents and primates based on ability of the isolated cells to reconstitute hematopoiesis in appropriate recipients (1–4). PSC capable of long-term (>4 mo) marrow reconstitution (5–7) were separated from cells capable of short-term (<1 mo) repopulation. Cell function correlated with the degree to which such PSC are in the G_0 stage of the cell cycle (8). Because cell quiescence correlates with the mitochondrial activity of the cells, the two cell populations are separable based on differences in their ability to take up the dye rhodamine 123 (Rh123) (9).

Many hematopoietic growth factors have been identified that affect the proliferation and differentiation of hematopoietic progenitor cells (10). In contrast, little is known about the factors that control proliferation of PSC.

Rh123-dull and Rh123-bright populations can proliferate and colonize murine marrow. Although both cell populations die within 24 hr in culture without growth factors, they can induce a cobblestone area of hematopoiesis if seeded on marrow-stromal cells (11, 12). The factors produced by the marrow stroma responsible for PSC growth have not yet been identified. Rh123-bright cells can be induced into cell cycle by interleukin 3 (IL-3) (13, 14). Erythropoietin (Epo) (15), macrophage colony-stimulating factor (M-CSF), IL-1, and IL-6 (16, 17) synergize with IL-3 by synchronizing entry of these cells into the cell cycle (16). However, proliferation of these cells results predominantly in differentiation because the cells with marrow-repopulating ability are lost after only a 24-hr exposure to IL-3 (15), whereas the number of progenitor cells in the culture progressively increases, reaching a peak after 4 days (18). Because IL-3 has not been shown to be produced by stromal cells *in vivo* or *in vitro*, the identity of the growth factor that induces proliferation of Rh123-bright cells remains to be determined.

Recently stem-cell factor (SCF), the ligand for the c-*kit* gene product (19–22), has been isolated, and its gene has been cloned and expressed. SCF is expressed in a number of tissues during early development (23) and potentially is involved in the organization of multiple tissues, including hematopoiesis. We have studied the effect of recombinant rat SCF, alone and in combination with other hematopoietic growth factors, on the proliferation and differentiation of murine progenitor cells as well as highly enriched Rh123-bright and Rh123-dull cells. Although SCF had little colony-stimulating activity by itself, it interacted with all growth factors tested and, in conjunction with IL-3, generated large numbers of CFC from Rh123-bright and Rh123-dull cells. Thus, SCF is the most potent stimulus identified to date for generating many CFC from pre-CFC.

MATERIALS AND METHODS

Mice. B6C3J (The Jackson Laboratory) or CD1 (Charles River Breeding Laboratories) males (10–12 g) were used. Mice were provided with sterilized food and water ad libitum.

Growth Factors. Pure recombinant murine IL-3 and granulocyte/macrophage CSF (GM-CSF) were provided by J. J. Mermod (Glaxo). Pure recombinant human Epo and granulocyte CSF (G-CSF) and purified recombinant rat SCF (24) were provided by J. Egrie, L. Souza, and K.Z. (Amgen), respectively.

Each growth factor was used at a concentration that induced a maximal number of colonies in serum-deprived

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CSF, colony-stimulating factor; SCF, stem cell factor; Rh123, rhodamine 123; CFC, colony-forming cells; PSC, pluripotent stem cells; IL, interleukin; Epo, erythropoietin; GM-, granulocyte/macrophage; M-CSF, macrophage CSF; FITC, fluorescein isothiocyanate; WGA, wheat germ agglutinin; G-CSF, granulocyte CSF; CFU-S, spleen colony-forming units; CFU-GM, GM colony-forming units; BFU-E, erythroid burst-forming cells; mAb, monoclonal antibody.

‡To whom reprint requests should be addressed at: New York Blood Center, 310 East 67th Street, New York, NY 10021.

cultures of total marrow. These concentrations were 100 units/ml for IL-3, 10 units/ml for GM-CSF, 1000 units/ml for G-CSF, 1.5 units/ml for Epo, and 100 ng/ml for SCF.

Enrichment of Hematopoietic Cells. Hematopoietic cells were enriched, as described (1, 25). Adult mouse marrow cells were collected by flushing the femurs with Hanks' balanced salt solution (GIBCO) buffered at pH 6.7 with Hepes (10 mM; Merck) and supplemented with penicillin (100 international units/ml) and streptomycin (0.1 mg/ml). Cells were filtered through a nylon sieve and then centrifuged (400 × *g* for 10 min at 4°C) in a discontinuous metrizamide (Nyegaard, Oslo) density gradient in the presence of wheat germ agglutinin (WGA) bound to fluorescein isothiocyanate (FITC; 1 μg/10 ml of metrizamide solution; Polysciences). The low-density cells were washed once and analyzed by using a light-activated cell sorter (FACS II; Becton Dickinson). Cells with medium and high WGA-FITC fluorescence, medium forward and low perpendicular light-scatter intensities, were sorted. Subsequently, WGA-FITC was removed from the cells by incubation with an isotonic solution of *N*-acetyl-D-glucosamine (0.2 mol/l; Polysciences). The cells were then labeled with the monoclonal antibody (mAb) 15-1.1 (26) directly conjugated with FITC and sorted again. The mAb 15-1.1-negative cells were stained with Rh123 (Eastman Kodak) and sorted again. Rh123-dull and -bright cells were separated on the basis of fluorescence. The effectiveness of separation was monitored by the spleen colony-forming assay (CFU-S). Preliminary experiments revealed a 1500-fold enrichment of day 12 CFU-S in the Rh123-bright population and a 250-fold increase in the Rh123-dull population (data not shown).

Cell Culture. Intact marrow cells or enriched cell populations were cultured either in semisolid medium to enumerate CFC, or 2.5 × 10^3 sorted cells were incubated in 0.5 ml of liquid culture for 2–21 days. In the latter case, the number of CFC in the culture was evaluated at different intervals by culturing an aliquot (20% of the volume) of cells from the suspension culture.

For CFC assays, 5 × 10^4 marrow cells were cultured in semisolid medium containing the following components in Iscove's modified Dulbecco's medium: methylcellulose (0.8%, final concentration), 2-mercaptoethanol (75 μM), and either 40% (vol/vol) fetal bovine serum (Hyclone) or a mixture of fetal bovine serum-replacing components—deionized bovine serum albumin (fraction V, Sigma) and bovine serum albumin-adsorbed cholesterol and soybean lecithin (Sigma; final concentration 200 μM for both), iron-saturated human transferrin (Behring; 9 μM), insulin (Sigma; 1.7 μM), nucleosides (10 μg/ml each), hemin (Sigma; 10 μM), sodium pyruvate (100 μM), and L-glutamine (2 mM), as reported (27, 28).

For liquid suspension cultures, methylcellulose was replaced by Iscove's modified Dulbecco's medium.

RESULTS

The Effect of SCF on CFC Growth. As shown in Fig. 1, increased concentrations of SCF interacted with Epo or G-CSF to promote the growth of erythroid bursts, granulocyte/macrophage (GM) colonies, or mixed-cell colonies. SCF, alone, supported the growth of few erythroid bursts; however, SCF synergized with Epo to provide more bursts than were obtainable with Epo plus spleen cell-conditioned medium (28). In contrast, SCF supported half the number of GM colonies found with G-CSF plus spleen cell-conditioned medium. SCF interacted with G-CSF in a dose-dependent fashion.

Although unable to promote mixed-cell colony growth by itself, SCF interacted with Epo to promote nearly as many

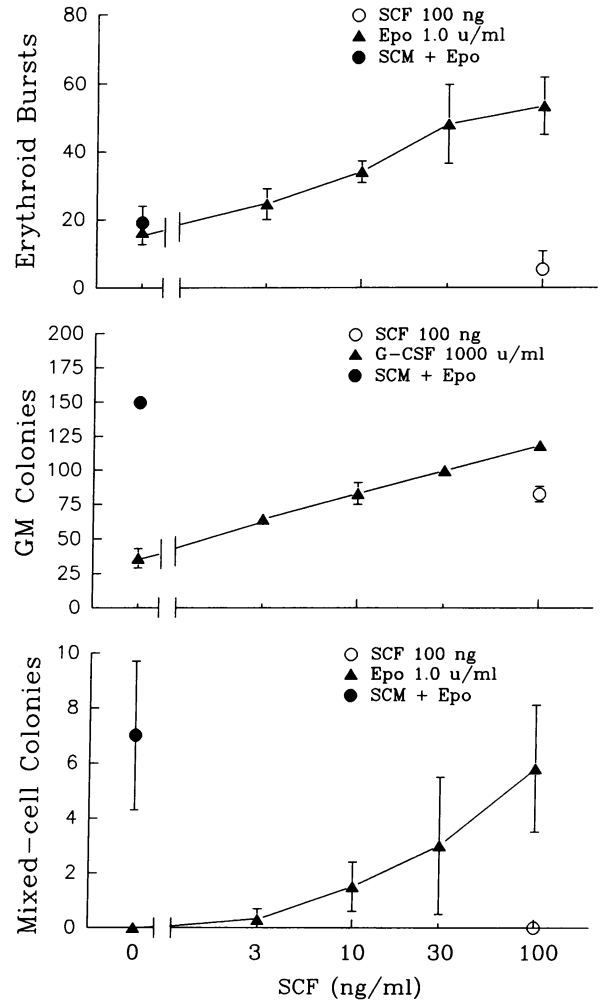

FIG. 1. Effect of increased concentrations of SCF on growth of erythroid bursts (*Top*), GM colonies (*Middle*), and mixed-cell colonies (*Bottom*) in serum-deprived cultures of unfractionated mouse marrow cells (5 × 10^4 cells per dish) in the presence of Epo or G-CSF. Number of colonies observed with 100 ng of SCF alone is also shown.

mixed-cell colonies as seen with Epo plus spleen cell-conditioned medium.

Fig. 2 shows the effect of SCF alone or in combination with other hematopoietic growth factors on colony formation by Rh123-bright cells. Erythroid bursts, GM colonies, and mixed-cell colonies were scored. SCF alone was incapable of supporting colony growth. IL-3, G-CSF, and GM-CSF, by themselves, supported only a few GM colonies. Epo alone supported no erythroid or mixed-cell colonies. In combinations, SCF with Epo gave rise to some erythroid bursts, whereas SCF, IL-3, and Epo, together, gave rise to 1–5 mixed-cell colonies, 50 GM colonies, and 5–10 pure erythroid bursts. SCF synergized with IL-3 to support GM colonies and synergized with G-CSF and GM-CSF to support the growth of GM colonies. Thus, SCF interacted with multiple hematopoietic growth factors to promote colony formation by a subpopulation of highly enriched murine hematopoietic cells.

Effect of SCF in Liquid Culture of Rh123-Bright and Rh123-Dull Cells. Fig. 3 shows the effect of SCF and IL-3, alone and in combination, on total cell numbers and CFC, over time, in serum-deprived liquid culture of Rh123-bright cells. The cell input was 2500 per well. After 2 days, the number of cells became virtually undetectable (Fig. 3*A*). Without growth factors, no cell proliferation occurred. In the presence of IL-3 alone, up to 30,000 cells were seen by day 5; this number declined by day 10. With SCF alone, several hundred cells

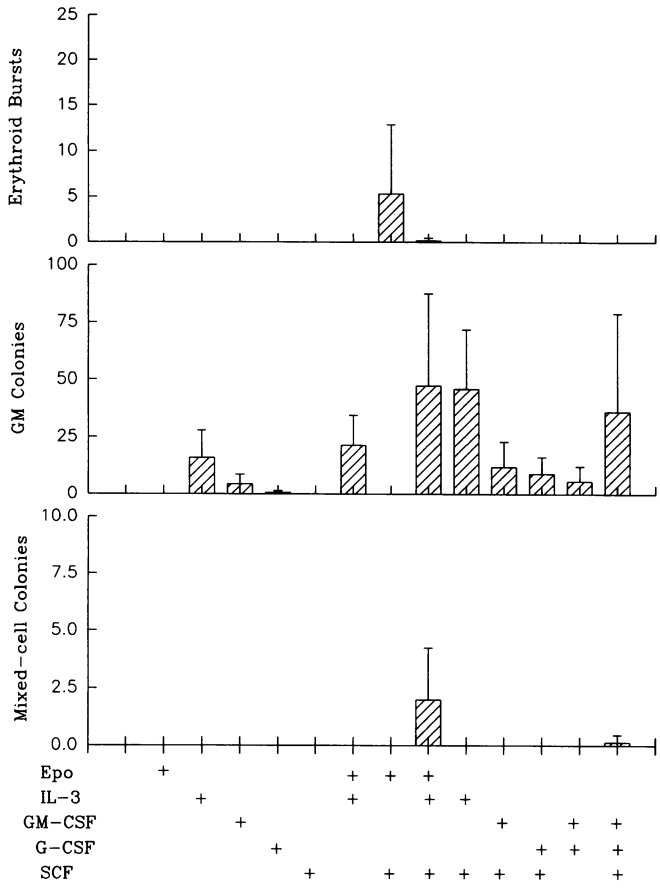

FIG. 2. Effect of SCF, alone or in combination with Epo, IL-3, GM-CSF, or G-CSF, on growth of erythroid bursts, GM colonies, and mixed-cell colonies (*Top*, *Middle*, or *Bottom*, respectively) from WGA^+/mAb 15-1.1$^-$/Rh123-bright marrow cells (500 cells per dish) in serum-deprived cultures. Data represent the mean (±SD) of three experiments performed in duplicate. Large SDs were seen due to the highly variable number of CFC detectable in primary cultures of Rh123-bright cells.

were present on day 5, but these became undetectable at day 10. IL-3 and SCF together, however, yielded nearly 200,000 cells by day 5, which declined to 100,000 by day 10.

Similar effects on CFC were seen (Fig. 3*B*). Few CFC were present in the Rh123-bright input cells. Without added growth factor, no CFC appeared. With IL-3, few GM colony-forming units (CFU-GM) were detected on day 2 but their numbers increased to ≈2000 by day 5. Some erythroid burst-forming cells (BFU-E) and mixed-cell CFC were detected by day 5. SCF alone gave rise to a small number of detectable BFU-E, GM-CFC, and mixed-cell CFC by day 5. Again, the combination of IL-3 and SCF was most dramatic in giving rise to 1500 mixed-cell CFC, nearly 15,000 GM-CFC, and over 700 BFU-E after 5 days in culture. CFC numbers declined by 10 days.

Subsequent experiments were done on day 5 cells to examine the interaction of SCF with other growth factors (Fig. 4). Again, without growth factors, cell numbers declined, and no CFC accumulated. SCF, G-CSF, and Epo, by themselves, promoted the accumulation of few CFC, and SCF and G-CSF maintained total cell numbers to a limited degree. In contrast, IL-3 and GM-CSF, by themselves, promoted considerable cell proliferation, and, for IL-3, the appearance of small numbers of BFU-E, GM-CFC, and mixed-cell CFC. GM-CSF is of particular interest in that it stimulated cell proliferation (≈100,000 from an input of 2500) but virtually no CFC. Most potent were SCF and IL-3; SCF and GM-CSF; and SCF, Epo, and IL-3. The addition of SCF

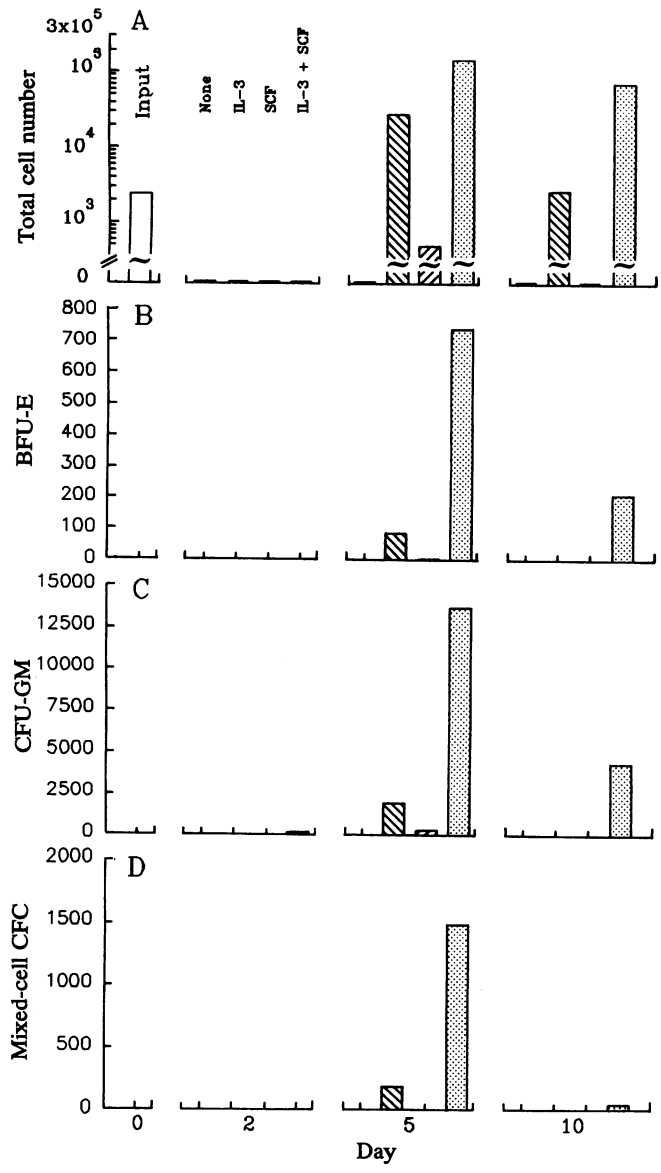

FIG. 3. Effect of SCF, alone or in combination with IL-3, on total cell numbers (*A*) or the number of BFU-E (*B*), CFU-GM (*C*), and mixed-cell CFC (*D*) arising in liquid culture of WGA^+/mAb 15-1.1$^-$/Rh123-bright marrow cells. Input cell values (day 0) and values after 2, 5, and 10 days are presented. Data represent the means of four separate experiments.

to the suspension culture resulted in much larger numbers of CFC of all types.

In experiments similar to those with Rh123-bright cells, 2500 Rh123-dull cells were placed into serum-deprived suspension culture. Results for 10 and 21 days of suspension culture are shown in Table 1, as no changes of consequence were seen during the first 5 days of culture. In these experiments, the Rh123-dull cells contained no detectable CFC at input. When no growth factors were added to the suspension cultures, no cells or CFC were recoverable. IL-3 alone, SCF alone, and the combination of G-CSF, GM-CSF, and IL-3 failed to sustain cell numbers or allow any accumulation of CFC. However, over a 10-day period, the combination of IL-3 and SCF not only maintained total cell numbers but also led to the appearance of BFU-E, GM-CFC, and mixed-cell CFC. These numbers increased further by day 21, at which point a nearly 8-fold amplification of total cell numbers was seen as well as more accumulation of BFU-E, GM-CFC, and mixed-cell CFC. By day 21, one-third of the cells were CFC.

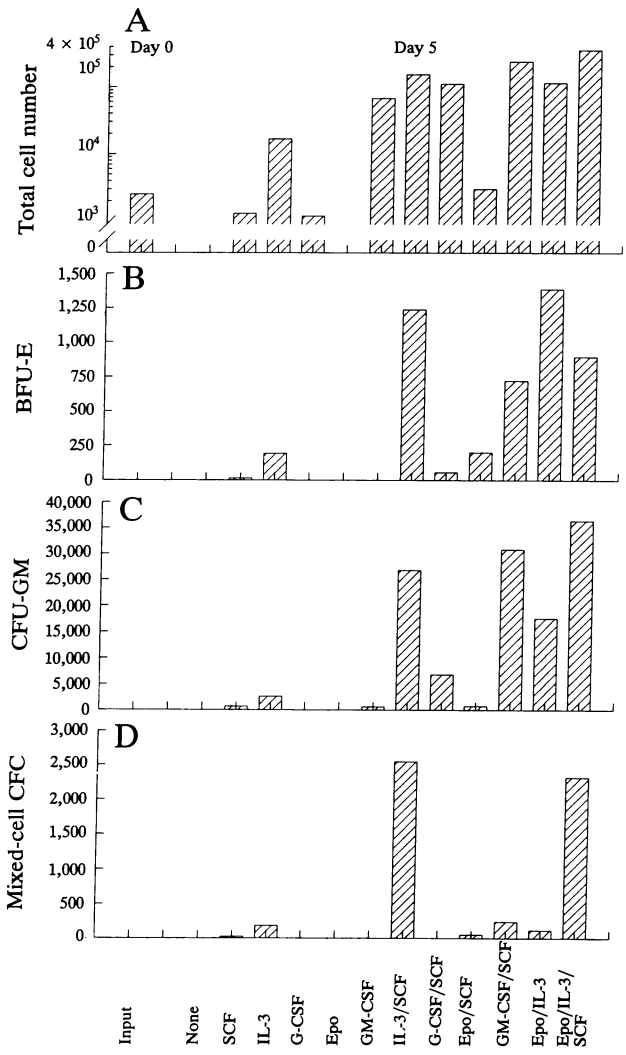

FIG. 4. Effect of SCF, alone and in combination with several hematopoietic growth factors, on proliferation and differentiation of WGA^+/mAb 15-1.1$^-$/Rh123-bright marrow cells after 5 days of liquid culture under serum-deprived conditions. Total cell number and numbers of BFU-E, CFU-GM, and mixed-cell CFC are shown in *A–D*, respectively. A representative experiment is shown.

DISCUSSION

SCF is an additional hematopoietic growth factor, the gene for which has recently been cloned and expressed (19–22, 24). SCF is encoded in the Steel locus and is the ligand for the c-*kit* tyrosine kinase receptor. Steel (Sl/Sl^d) mice have a number of developmental abnormalities, including altered coat color, lack of primordial germ cells, reduced support of hematopoietic stem cells, lack of mast cells, as well as macrocytic anemia (29). SCF is expressed differentially by a variety of tissues during murine embryogenesis and may have a role in development (23).

We have studied the effect of purified recombinant rat SCF on the *in vitro* proliferation and differentiation of adult murine hematopoietic progenitor cells and have examined the effect of SCF, alone or with other hematopoietic growth factors, on the proliferation and differentiation of highly enriched murine progenitor cells. These studies were designed to assess the range of progenitor cell activities of SCF and the range of potential interactions that SCF might have with other growth factors. The enrichment procedure chosen was designed to isolate populations of cells that had been shown previously to contain within them the cells responsible for short- or long-term marrow repopulation (Rh123-bright and Rh123-dull, respectively).

Table 1. Effect of hematopoietic growth factors alone and in combination on proliferation and differentiation of Rh123-dull cells

Factor(s) added	Total cells/ml	BFU-E	GM-CFC	Mixed-cell CFC
Day 0	2,500	0	0	0
Day 10				
None	0	0	0	0
IL-3	0	0	0	0
SCF	0	0	0	0
IL-3 + SCF	2,770	25	982	31
G-CSF + GM-CSF + IL-3	0	0	0	0
Day 21				
None	0	0	0	0
IL-3	0	0	0	0
SCF	0	0	0	0
IL-3 + SCF	18,500	185	5,720	69
G-CSF + GM-CSF + IL-3	0	0	0	0

SCF alone was found to have little colony-stimulating activity in serum-deprived cultures. These results are similar to those of Broxmeyer *et al.* (30). Our findings are also similar to those made earlier for GM-CSF and IL-3 (15, 31) and are consistent with the thesis that early-acting factors require one or more later-acting factors for full colony growth to be observed. This hypothesis was borne out in studies that documented various patterns of interactions between SCF and IL-3, GM-CSF, G-CSF, and Epo.

In subsequent experiments, murine marrow cells were separated into Rh123-bright and Rh123-dull populations. Rh123-bright or Rh123-dull cells gave rise to few or no colonies when plated directly in semisolid medium with growth factors and without serum. Previous studies have demonstrated that both populations give rise to spleen colonies in the CFU-S assay. Rh123-bright cells contain both day 8 and day 12 CFU-S, whereas Rh123-dull cells contain predominantly day 12 CFU-S (11). Rh123-bright cells are incapable of long-term marrow reconstitution, suggesting that cells other than day 12 CFU-S, present among the Rh123-dull cells, are responsible for this function.

The results observed with the enriched cell populations confirmed that SCF can interact with a variety of hematopoietic growth factors and suggested that SCF was key to the accumulation of CFC over time. The results also demonstrated that the kinetics of response to SCF and other growth factors of Rh123-bright cells differed from the kinetics of the response of Rh123-dull cells. The peak response of Rh123-bright cells was on day 4 or 5 of suspension culture. For Rh123-dull cells, there was no early wave of proliferation and, under the conditions tested, the highest numbers of cells were seen at day 21. At this time, one-third of all cells that had accumulated were CFC of various classes, including mixed-cell CFC.

The kinetics of response to SCF in conjunction with IL-3 suggest that the more primitive cells are among the Rh123-dull population, in keeping with observations that this cell population is responsible for long-term marrow reconstitution. Furthermore, the results raise the possibility that SCF, alone or with one or another growth factor, can amplify the population giving rise to long-term marrow reconstitution.

The results also suggest other uses for SCF. If Rh123-dull cells can be activated into cell cycle by SCF, they provide a more prime target for gene-transfer experiments. Furthermore, if cells capable of long-term marrow reconstitution are amplified by SCF, strategies may be devised for the amplification of human stem cells *in vitro* and their storage for

eventual transplantation, because the gene for human SCF has also been cloned and expressed (24). The successful development of this methodology would simplify the transplantation process in a number of ways.

The excellent secretarial support of Ms. J. Pack and technical skills of Mr. Harold Ralph are gratefully acknowledged. This study was supported by Research Grants DK-41937 and HL-46524 from the National Institutes of Health, Department of Health and Human Services, institutional funds of the New York Blood Center, and Progetto Finalizzato Ingegneria Genetica.

1. Visser, J. W. M., Bauman, J. G. J., Mulder, A. H., Eliason, J. F. & de Leeuw, A. M. (1984) *J. Exp. Med.* **59,** 1576–1590.
2. Visser, J. W. M. & Van Bekkum, D. W. (1990) *Exp. Hematol. (N.Y.)* **18,** 248–256.
3. Spangrude, G. J., Heimfeld, S. & Weissman, I. L. (1988) *Science* **241,** 58–62.
4. Berenson, R. J., Andrews, R. G. & Bensinger, W. I. (1988) *J. Clin. Invest.* **81,** 951–955.
5. Jones, R. J., Wagner, J. E., Celano, P., Zicha, M. S. & Sharkis, S. J. (1990) *Nature (London)* **347,** 188–189.
6. Spangrude, G. J. & Johnson, G. R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 7433–7437.
7. Micklem, H. S., Lennon, J. E., Ansell, J. D. & Gray, R. A. (1987) *Exp. Hematol. (N.Y.)* **15,** 251–257.
8. Hodgson, G. S. & Bradley, T. R. (1979) *Nature (London)* **281,** 381–382.
9. Johnson, L. V., Walsh, M. L., Bockus, B. J. & Chen, L. B. (1981) *J. Cell Biol.* **88,** 526–535.
10. Cross, M. & Dexter, T. M. (1991) *Cell* **64,** 271–280.
11. Visser, J. W. M., Hogeweg-Platenburg, M. G. C. & de Vries, P. (1990) in *The Biology of Hematopoiesis*, eds. Dainiak, N., Cronkite, E. P., McCaffrey, R. & Shadduck, R. (Wiley/Liss, New York), pp. 1–8.
12. Van der Sluijs, J. P., de Jong, J. P., Brons, N. H. C. & Ploemacher, R. E. (1990) *Exp. Hematol. (N.Y.)* **18,** 893–896.
13. Migliaccio, A. R. & Visser, J. W. M. (1986) *Exp. Hematol. (N.Y.)* **14,** 1043–1048.
14. Muller-Sieburg, C. E., Townsend, K., Weissman, I. L. & Rennick, D. (1988) *J. Exp. Med.* **167,** 1825–1840.
15. Migliaccio, G., Migliaccio, A. R. & Visser, J. W. M. (1988) *Blood* **72,** 944–951.
16. Dexter, T. M., Heyworth, C. M., Spooncer, E. & Ponting, I. (1990) *Philos. Trans. R. Soc. London Ser. B* **327,** 85–98.
17. Ikebuchi, K., Wong, G. G., Clark, S. C., Ihle, J. N., Hirai, Y. & Ogawa, M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9035–9039.
18. Iscove, N. N. & Yan, X.-Q. (1990) *J. Immunol.* **145,** 190–195.
19. Williams, D. E., Eisenman, J., Baird, A., Rauch, C., Van Ness, K., March, C. J., Park, L. S., Martin, U., Mochizuki, D. Y., Boswell, H. S., Burgess, G. S., Cosman, D. & Lyman, S. D. (1990) *Cell* **63,** 167–174.
20. Flanagan, J. G. & Leder, P. (1990) *Cell* **63,** 185–194.
21. Zsebo, K. M., Williams, D. A., Geissler, E. N., Broudy, V. C., Martin, F. H., Atkins, H. L., Hsu, R.-Y., Birkett, N. C., Okino, K. H., Murdock, D. C., Jacobsen, F. W., Langley, K. E., Smith, K. A., Takeishi, T., Cattanach, B. M., Galli, S. J. & Suggs, S. V. (1990) *Cell* **63,** 213–224.
22. Nocka, K., Buck, J., Levi, E. & Besmer, P. (1990) *EMBO J.* **9,** 3287–3294.
23. Matsui, Y., Zsebo, K. M. & Hogan, B. L. M. (1990) *Nature (London)* **347,** 667–669.
24. Martin, F. H., Suggs, S. V., Langley, K. E., Lu, H. S., Ting, J., Okino, K. H., Morris, C. F., McNiece, I. K., Jacobsen, F. W., Mendiaz, E. A., Birkett, N. C., Smith, K. A., Johnson, M. J., Parker, V. P., Flores, J. C., Patel, A. C., Fisher, E. F., Erjavec, H. O., Herrara, C. J., Wypych, J., Sachdev, R. K., Pope, J. A., Leslie, I., Wen, D., Lin, C.-H., Cupples, R. L. & Zsebo, K. M. (1990) *Cell* **63,** 203–211.
25. Visser, J. W. M. & de Vries, P. (1988) *Blood Cells* **14,** 369–384.
26. Visser, J. W. M. & de Vries, P. (1990) *Methods Cell Biol.* **33,** 451–468.
27. Migliaccio, G. & Migliaccio, A. R. (1987) *Br. J. Haematol.* **67,** 129–133.
28. Migliaccio, G. & Migliaccio, A. R. (1987) *Ann. N.Y. Acad. Sci.* **511,** 39–49.
29. Sarvella, P. A. & Russell, L. B. (1956) *J. Hered.* **47,** 123–128.
30. Broxmeyer, H. E., Hangoc, G., Cooper, S., Anderson, D., Cosman, D., Lyman, S. D. & Williams, D. E. (1991) *Exp. Hematol. (N.Y.)* **19,** 143–146.
31. Migliaccio, G., Migliaccio, A. R., Kaushansky, K. & Adamson, J. W. (1989) *Exp. Hematol. (N.Y.)* **17,** 110–115.