Stimulating Spectrum of Human Recombinant Multi-CSF (IL-3) on Human Marrow Precursors: Importance of Accessory Cells

By Fredrik J. Bot, Lambert Dorssers, Gerard Wagemaker, and Bob Löwenberg

Recently, human multi-CSF was obtained by molecular cloning. In the present study, the effects of multi-CSF in vitro were investigated by comparative culture of whole bone marrow or progenitor cells obtained by sorting the cell fraction that binds the monoclonal antibody (MoAb) BI3C5 (CD 34). Multi-CSF stimulated erythroid (BFU-E), multipotential (CFU-GEMM) and eosinophil (CFU-Eo) colonies in cultures of the progenitor cell enriched fraction, whereas (besides BFU-E, CFU-GEMM, and CFU-Eo) granulocyte (CFU-G), granulocyte-macrophage (CFU-GM), and macrophage (CFU-M) colony-forming cells also were stimulated by multi-CSF when unfractionated bone marrow was cultured. Reconstitution of the progenitor cell fraction (BI3C5 positive) with the BI3C5-negative population restored the broad spectrum of progenitor cell stimulation. This suggested that accessory cells are required for expression of the full spectrum of progenitor cell stimulation by multi-CSF. Subsequently, specific marrow cell populations, including T lymphocytes, granulocytic cells, and monocytes, were prepared by using selected MoAbs in complement-mediated lysis or cell sorting, added to cultures of hematopoietic progenitors and tested for accessory cell function. The results demonstrate that small numbers of monocytes permit the stimulation of CFU-G, CFU-GM, and CFU-M by multi-CSF. These monocyte-dependent stimulating effects on CFU-G, CFU-GM, and CFU-M could also be achieved by adding recombinant GM-CSF as a substitute for monocytes to the cultures. Therefore, multi-CSF most likely has direct stimulative effects on BFU-E, CFU-GEMM, and CFU-Eo and indirect effects on CFU-G, CFU-GM, and CFU-M in the presence of monocytes.

THE PROLIFERATION and differentiation of hematopoietic cells is regulated by specific growth factors.[1] The genes encoding several human hematopoietic growth factors have been molecularly cloned and expressed in suitable host systems to produce the recombinant growth factors.[2-6] The murine growth factor interleukin-3 (IL-3), has been described as a proliferative stimulus for early progenitors in the mouse, including the multipotential progenitors and those of granulocytes and macrophages, erythrocytes, eosinophilic granulocytes, megakaryocytes, and mast cells.[7-9] Recently, a homologous human growth factor (multilineage colony-stimulating factor, multi-CSF) was discovered by cDNA cloning.[10,11] Human multi-CSF stimulates erythroid, myeloid, and multipotential hematopoietic progenitor cells.[10] Hence, the spectrum of stimulation of this molecule resembles that of murine IL-3. In the experiments we present, we provide further insight into the stimulative effects of multi-CSF on human hematopoietic progenitor cells in vitro.

MATERIALS AND METHODS

Preparation of cell suspension. Bone marrow was obtained by posterior iliac crest puncture from hematologically normal adults who had given their informed consent. A separate donor was used for each experiment. The marrow was collected in Hanks' balanced salt solution (HBSS) with heparin, diluted in HBSS and layered over a Ficoll-gradient (1.077 g/cm^2; Nycomed, Oslo). After centrifugation, the mononuclear cells were harvested, washed twice in HBSS and resuspended in phosphate-buffered saline (PBS) with 2% heat-inactivated fetal calf serum (FCS).

Recombinant human CSFs. The preparation of recombinant human multi-CSF has been described in detail elsewhere.[10] In brief, mRNA was prepared from activated human lymphocytes and used for cDNA synthesis. The cDNA clone was identified by hybridization with mouse IL-3 cDNA. This cDNA was then inserted into a eukaryotic expression vector (pLB4) and transfected into monkey COS cells, which were then cultured for 48 to 72 hours. The resulting conditioned medium [COS(pLB4)CM] was used in bone marrow colony assay and is designated "multi-CSF." Medium conditioned by COS cells transfected with the vector without the insert encoding multi-CSF did not stimulate colony formation in cultures of human bone marrow cells.[10] Recombinant human G-CSF[4,5] from Genetics Institute (Cambridge, MA) and recombinant GM-CSF[2,3] from Biogen SA (Geneva) were used at optimal concentrations of 1:1,000 and 1,000 U/mL, respectively.

Labeling and cell sorting. A cell sample was incubated with the monoclonal antibody (MoAb) BI3C5 (CD 34; Sera-lab, Crawley Down, England)[12] at a final dilution of 1:100 in PBS with 5% FCS for 30 minutes on ice. After being washed in PBS and 5% FCS, the cells were further incubated with goat anti-mouse–FITC (GAM-FITC, Nordic, Tilburg, The Netherlands) at a dilution of 1:40 for another 30 minutes on ice. The cells were then washed twice and resuspended in PBS at a concentration of 10^6 nucleated cells/mL. Control cells were incubated with GAM-FITC alone. Analysis and cell sorting were performed under sterile conditions with a FACS 440 (Becton Dickinson, Sunnyvale, CA) at a maximum rate of 2,000 cells/second. The separation between positive and negative fractions was done so that the BI3C5 positive cell fraction regularly contained 3% to 4% of the total nucleated cell number. This resulted in a cell population enriched for blast cells and hematopoietic progenitors but depleted of mature erythroid and myeloid cells and T lymphocytes. In certain experiments, the BI3C5 negative fraction was incubated with VIM-2 (IgM, reactive with myelomonocytic cells, final dilution 1:50),[13] or T3 (IgG2, CD3, mature T lymphocytes, final dilution 1:10), or B4.3 (IgM, CD15, myeloid cells; final dilution 1:500),[14] or B44.1 (IgM, CD14 monocytes; final dilution 1:20)[15] and stained with GAM-FITC, after which the positive cells were sorted and added to the cultures. The number of these cells added to the enriched BI3C5 positive cells in culture reconstituted the original

From the Dr Daniel den Hoed Cancer Center and Rotterdam RadioTherapeutic Institute; and the Radiobiological Institute TNO, Rijswijk, and Department of Radiobiology, Erasmus University, Rotterdam.

Submitted August 4, 1987; accepted January 25, 1988.

Address reprint requests to B. Löwenberg, PhD, The Dr Daniel den Hoed Cancer Center, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.

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. section 1734 solely to indicate this fact.*

0006-4971/88/7106-0009$3.00/0

numerical proportions of the two cell populations in the Ficoll-fractionated marrow specimen unless stated otherwise.

Complement-mediated cytolysis. In several experiments, we used a different approach for the addition of subfractions from the BI3C5-negative cell fraction. After sorting, the BI3C5-negative cells were incubated with either MoAb VIM-2 or T3 at optimal concentrations (30 minutes on ice) and then incubated with rabbit complement at a final concentration of 40% (30 minutes at 25°C) and washed twice. These VIM-2– or T3-depleted BI3C5-negative cells were then added to the appropriate cultures and tested for accessory abilities.

CFU-GEMM culture assay. Mixed colonies were grown as described before by Fauser and Messner with slight modifications.[16,17] Sorted or unsorted marrow cells were cultured in a 1-mL mixture of Iscove's modified Dulbecco's medium (IMDM), 1.1% methylcellulose, 30% autologous heparinized plasma, BSA, transferrin, lecithin, sodium-selenite and 2-mercaptoethanol. Cells were added at a concentration of 8×10^4/mL in total marrow cultures and 0.2 to 0.5×10^4/mL for the BI3C5-positive cell fractions. Exogenous growth stimuli were added in the form of various concentrations of multi-CSF and recombinant human erythropoietin (Epo) at a concentration of 1 U/mL (Kirin-Amgen, Thousand Oaks, CA). Cultures with 10% of a medium stimulated by leukocytes in the presence of 1% phytohemagglutinin (PHA-LCM),[18] as based on the original CFU-GEMM assay, were done for comparison. Dishes were incubated at 37°C and 100% humidity in an environment of 5% CO_2 in air. Colonies were scored at day 15 and identified by their distinct morphological appearance at 100× magnification. Numbers of colonies refer to the means of duplicate cultures. In selected cases, the nature of the colonies was verified cytologically after they had been plucked from the plates with a finely drawn Pasteur pipette, and stained with May-Grünwald-Giemsa. Mixed colonies were always verified cytologically. Megakaryocyte colonies were not assayed since CFU-Meg could not be detected reproducibly in every bone marrow sample. However, ~15% of CFU-GEMM in the multi-CSF–stimulated cultures contained megakaryocytes.

RESULTS

Multi-CSF as a stimulator of purified hematopoietic progenitor cells. To minimize a possible interference of nonclonogenic accessory cells, we enriched progenitor cells from normal human bone marrow by sorting the cell fraction positive for MoAb BI3C5 (CD 34), and cultured the cells at low cell concentrations (2 to 5×10^3/mL) in the presence of multi-CSF. The results of a representative experiment are shown in Fig 1. When Epo was added as the only exogenous growth-stimulating factor some background erythroid bursts (BFU-E) were formed. BFU-E numbers rose markedly when graded concentrations of multi-CSF were added and reached a plateau at 1% to 3% (vol/vol) multi-CSF. In subsequent experiments, a concentration of 3% multi-CSF was used. Eosinophil (CFU-Eo) and multipotential (CFU-GEMM) colonies appeared in multi-CSF–stimulated cultures, and their numbers rose as a function of increasing concentrations of multi-CSF. When only multi-CSF but no Epo was added to the cultures, neither red nor mixed colonies appeared, whereas the number of eosinophil colonies remained constant. A remarkable number of eosinophil colonies was formed in the presence of multi-CSF with or without Epo. Multi-CSF did not stimulate significant numbers of granulocyte (CFU-G), granulocyte-macrophage (CFU-GM), or macrophage (CFU-M) colonies. The numbers of BFU-E obtained with multi-CSF were similar to those stimulated by a crude conditioned medium (PHA-LCM), but somewhat lower numbers of CFU-GEMM and CFU-Eo were obtained with multi-CSF than with PHA-LCM. In several experiments with a greater concentration of multi-CSF (10% vol/vol), the colony numbers, in particular those of BFU-E, declined. This reduction suggests the presence of inhibitory factors in the multi-CSF preparation (COS supernatant). Recently, we used partially purified *Escherichia coli*-derived multi-CSF in concentrations up to 30 times (expressed as activity) greater than the maximal concentration of COS-supernatant multi-CSF, and demonstrated no inhibitory effect. The pattern of stimulation, ie, induction of colony formation from BFU-E, CFU-Eo, and CFU-GEMM, was identical for both types of multi-CSF.

Role of accessory cells. To examine a possible role of BI3C5-negative accessory cells in the stimulatory effects of multi-CSF, we compared the effects of multi-CSF on: (a) a mock-sorted cell fraction, ie, passed through the cell sorter without selecting for a specific marker (total marrow nucleated cells) (Fig 2A); (b) the sorted BI3C5-positive cell population (precursor cell fraction) (Fig 2B); and (c) the BI3C5-positive cell fraction supplemented with the number of BI3C5-negative cells that reconstituted the total marrow cell population (Fig 2C). Multi-CSF appeared to stimulate not only BFU-E, CFU-GEMM, and CFU-Eo but also CFU-G, CFU-GM and CFU-M in unfractionated bone marrow cells (Fig 2A). This contrasts with the pattern of

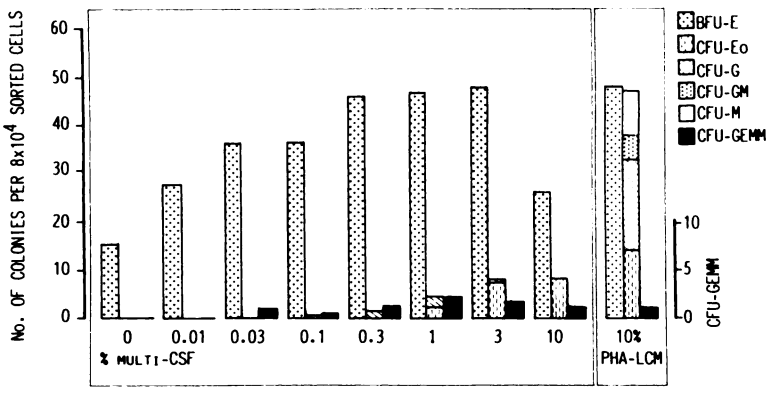

Fig 1. Colony formation in response to multi-CSF: culture of purified human hematopoietic progenitors. Numbers of erythroid, multipotential, and four classes of myeloid colonies (Eo, G, GM, M) in vitro are plotted as a function of increasing concentrations of recombinant multi-CSF. The results from one representative experiment are shown. The BI3C5 (CD34)-positive fraction was obtained by cell sorting from 8×10^4 mononuclear marrow cells and represented 5% of the original cell population. Values represent mean colony counts of duplicate cultures. Epo (1 U/mL) was added to all cultures; in cultures without Epo and without multi-CSF no colonies appeared. Colony growth after stimulation with 10% PHA-LCM is shown for comparison. Data for one of duplicate experiments are shown.

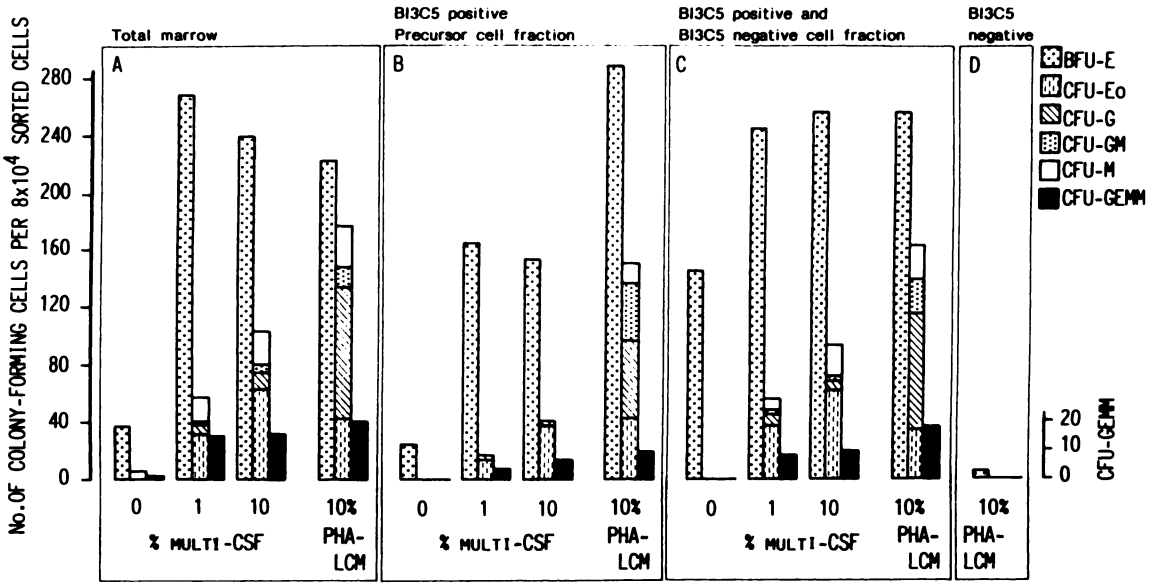

Fig 2. Colony formation in response to multi-CSF: direct comparison between total human bone marrow and purified progenitor cells. Different cell populations prepared from human marrow were cultured. (A) Total mononuclear marrow cells (separated on Ficoll-Isopaque) were mock-sorted and cultured at 8 × 10^4 cells/dish. (B) The progenitor cell fraction (BI3C5 positive) was cultured at 4 × 10^3 cells/dish. (C) The sorted BI3C5-positive fraction was reconstituted with the original proportion of BI3C5-negative cells and then cultured. (D) The sorted BI3C5-negative fraction plated in culture with Epo plus PHA-LCM. Colony numbers are plotted as a function of stimulation with various concentrations of multi-CSF, ie, 0%, 1%, and 10% (vol/vol). Control cultures with 10% PHA-LCM were run in parallel; results are included in A through C. All cultures also contained Epo (1 U/mL). Cultures with no Epo and no multi-CSF did not support any colony growth (data not given). Data for one of duplicate experiments are shown.

stimulation in the BI3C5-positive precursor cell fraction (Figs 1 and 2B): ie, stimulation of BFU-E, CFU-GEMM, and CFU-Eo only, but not the other myeloid colony-forming cells (CFU-G, CFU-GM, CFU-M) (Fig 2C). The addition of BI3C5-negative cells to the BI3C5-positive cell fraction completely restored the stimulative effect of multi-CSF on CFU-G, CFU-GM, and CFU-M. The BI3C5-negative cell fraction per se did not contain significant numbers of colony-forming cells (Fig 2D). Because these experimental data suggested that CFU-G, CFU-GM, and CFU-M colony formation in response to multi-CSF depended on the presence of BI3C5-negative cells or a subpopulation of these in culture, we subsequently examined the effect of the removal of specific subpopulations from this cell fraction using complement-mediated cytolysis of VIM-2 (myelomonocytic) or T3 (mature T lymphocytes) positive cells (Fig 3). VIM-2 lysis abrogated the augmentory effect of the BI3C5 negative cells whereas T3 lysis did not. These results suggested that

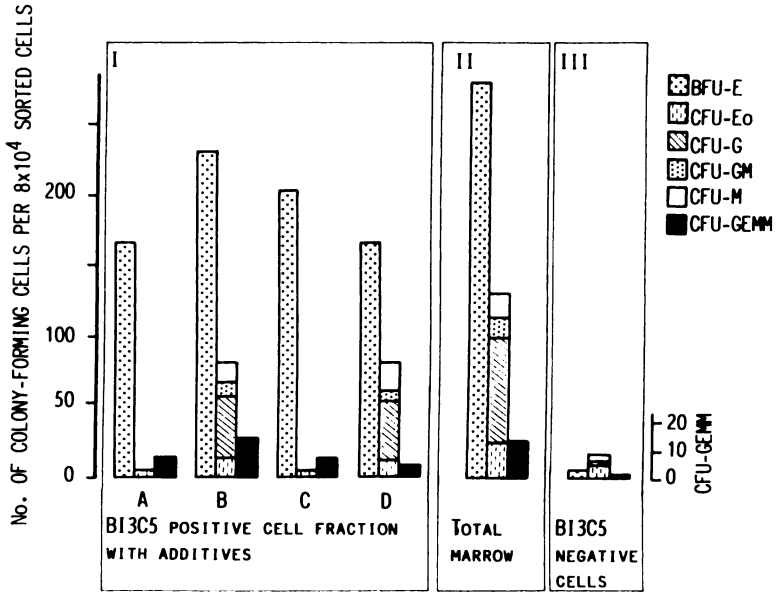

Fig 3. Colony formation in response to multi-CSF: addition of accessory cells to hematopoietic progenitor cells. Different subsets of cells were added in vitro and tested for accessory functions in cultures of the purified hematopoietic progenitor cell fraction (BI3C5 positive) that contained Epo and 3% multi-CSF. (I) Purified progenitor cell fraction (BI3C5 positive), 2.4 × 10^3 cells/dish with various cell supplements. (A) No accessory cells added. (B) Addition of BI3C5-negative cells (7.8 × 10^4 cells/dish). (C) Addition of BI3C5-negative cells depleted of Vim-2-positive cells following complement-mediated lysis. (D) Addition of BI3C5-negative cells depleted of T3-positive cells following complement-mediated lysis. Colony data from the total marrow population (II) and the BI3C5-negative fraction (III) are given for comparison. Data for one of duplicate experiments are plotted.

the active accessory cells are VIM-2 positive and thus belong to the myelomonocytic lineage.

Following an alternative approach, specific VIM-2, T3 (mature T lymphocytes), B44.1 (monocytes), and B4.3 (granulocytes) positive subsets were obtained from the BI3C5-negative fraction by cell sorting (Fig 4) and examined for their abilities to exert the accessory cell effects. Supplementation of VIM-2–positive or B44.1-positive cells to the BI3C5-positive progenitor cell fraction restored the development of colonies originating from CFU-G, CFU-GM and CFU-M in cultures stimulated with multi-CSF. As few as 2.5×10^4 VIM-2 or 0.5×10^4 B44.1 surface marker-positive cells were capable of enhancing colony growth. However, 2×10^4 B4.3-positive granulocytes or T3-positive lymphocytes were ineffective. Indeed, CFU-GEMM, BFU-E, and CFU-Eo were also slightly susceptible to the potentiating effect of monocytes, at least when large numbers of monocytes were added (Fig 4G).

We examined whether the effect of the addition of monocytes to cultures of the BI3C5-positive progenitor cell fraction to evoke CFU-G, CFU-GM, and CFU-M colony formation could be mimicked by supplementing exogenous GM-CSF or G-CGF (instead of monocytes) to the multi-CSF cultures (Fig 5). When G-CSF or GM-CSF were added to multi-CSF cultures of purified bone marrow progenitors, additional CFU-G or CFU-G, CFU-GM and CFU-M were induced to colony formation. There was no evidence for a synergistic effect between these factors. When all three factors (ie, multi-CSF, GM-CSF, and G-CSF) were included in culture, no significant further increase in colony formation was seen above the level of multi-CSF plus GM-CSF stimulation. This indicates that multi-CSF and GM-CSF in conjunction provide optimal stimulation of CFU-G, CFU-GM, and CFU-M from the BI3C5-positive progenitor cell fraction and that GM-CSF can substitute the monocyte accessory cell effect.

DISCUSSION

We assessed the stimulating abilities of the recombinant human growth factor multi-CSF. In cultures of enriched human hematopoietic progenitor cells (based on BI3C5 reactivity), multi-CSF stimulated BFU-E, CFU-Eo, and CFU-GEMM. In the presence of BI3C5-negative cells, the spectrum of multi-CSF stimulation was broader and also included CFU-G, CFU-GM, and CFU-M. These data suggest a direct stimulative effect of multi-CSF on BFU-E, CFU-Eo, and CFU-GEMM and an effect on CFU-G, CFU-GM, and CFU-M in the presence of a secondary cell. Experiments based on the addition of specific subsets of cells (selected by cell sorting) as well as elimination of these subsets from the accessory cell fraction (by complement-mediated lysis) identified a VIM-2 and B44.1 surface marker-positive cell population as the active accessory cell. The accessory cell function could not be attributed to the subsets that expressed the T-lymphocytic T3 or granulocytic B4.3 markers. The VIM-2 and B44.1-positive surface phenotype of the accessory cells demonstrates the monocytic identity of the cells. It became apparent that the accessory cell phenomenon was cell dose dependent and that minimal numbers (0.5×10^4 per dish) of these monocytic cells were sufficient to allow the outgrowth of CFU-G, CFU-GM, and CFU-M in multi-CSF–stimulated cultures. The addition of four times the proportional number of VIM-2–positive cells or B44.1-positive cells to purified progenitors further elevated GM and M colony numbers, and also raised the number of BFU-E,

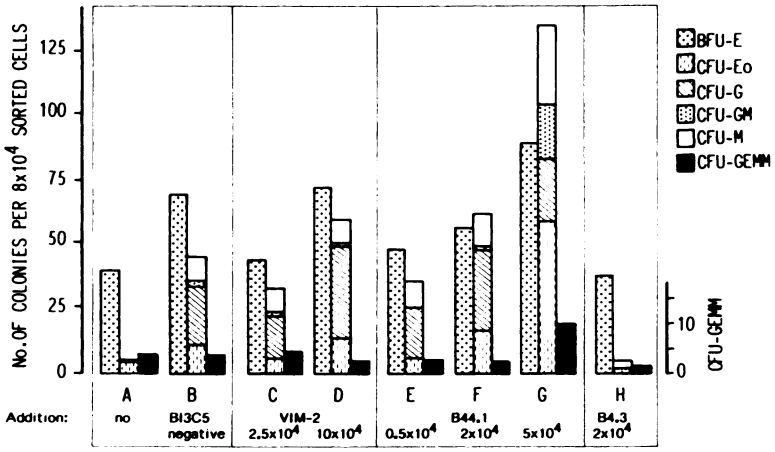

Fig 4. Colony formation in response to multi-CSF: effect of addition of different subpopulations of accessory cells. Purified hematopoietic progenitor cells (BI3C5 positive; 4×10^3 cells/dish) were plated in with 3% multi-CSF and Epo. To these cultures VIM-2–positive, B44.1-positive, B4.3-positive, or T3-positive cells were added and examined for enhancement of colony formation. The VIM-2-, B44.1-, B4.3-, and T3-positive cells were separated by cell sorting from the BI3C5-negative cell fraction. (A) Progenitor cells with no addition of accessory cells. (B) Addition of BI3C5-negative cells (7.6×10^4 cells/dish). (C and D) Addition of VIM-2–positive cells (promyelocytes to granulocytes and monocytes) (C, 2.5×10^4 and D, 1×10^5 cells/dish). (E through G) Addition of the B44.1-positive cells (monocytes) in numbers that were originally present in the BI3C5-negative accessory cell population (E, 0.5×10^4 cells/dish) and increasing numbers (F, 2×10^4 and G, 5×10^4 cells/dish). (H) Addition of the B4.3-positive cells (metamyelocytes to granulocytes) in original numbers (2×10^4 cells/dish). Results of the supplemented T3-positive cells were identical to those of the B4.3-positive cells (not shown). Numbers of colonies grown from the separate Vim-2–positive, B44.1-positive, or B4.3-positive fractions were: BFU-E, 2-4-1; CFU-G, 0-1-0; CFU-Eo, 1-1-0; CFU-M, 0-1-0; CFU-GM, 1-0-0; CFU-GEMM, 0-0-0. Results of one of three repeat experiments are shown.

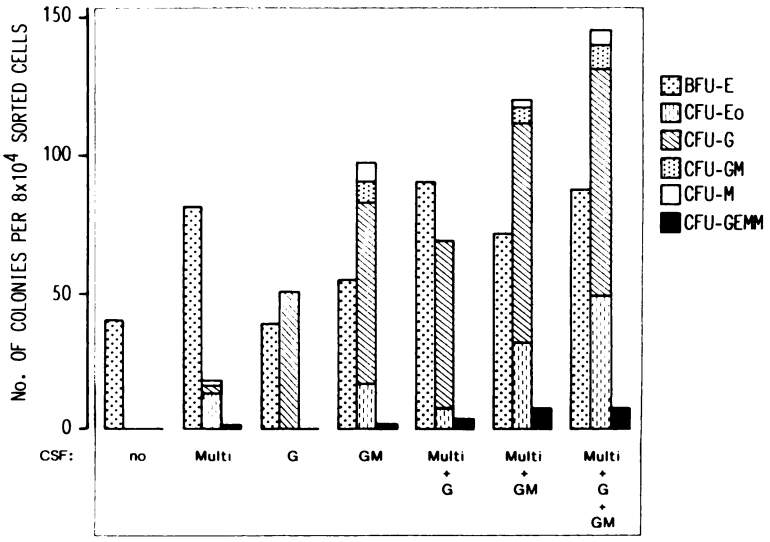

Fig 5. Colony formation from purified marrow cells in response to multi-CSF, G-CSF, and GM-CSF. Hematopoietic progenitor cells (BI3C5 positive, 4.5 × 10^3 cells/dish) were plated with multi-CSF, G-CSF, and/or GM-CSF; all cultures contained Epo. Results are those of one of two repeat experiments.

CFU-Eo, and CFU-GEMM. Three possible mechanisms through which monocytes enhance growth of these colony types stimulated by multi-CSF can be proposed: (a) production of growth factors (eg GM-CSF or M-CSF) by monocytes in response to multi-CSF, (b) production of growth factors by monocytes independent of multi-CSF, and (c) cell–cell interactions between monocytes and colony-forming cells. The present experiments do not allow a distinction between the alternative mechanisms for the enhancement of CFU-G, CFU-GM, and CFU-M colony growth. The possibility that monocytes produce growth factors that can induce CFU-G, CFU-GM, and CFU-M would at least be consistent with the observation that monocytes can be stimulated to produce colony-stimulating factors.[19] More recently, specific evidence was obtained demonstrating that monocytes may produce M-CSF, interferon (IFN), tumor necrosis factor (TNF), and GM-CSF.[20,21] This could explain our results showing that the addition of recombinant GM-CSF could fully substitute the monocyte effect and that G-CSF could partly replace the role of the monocytes in the multi-CSF cultures, thereby resulting in the induction of G, GM, and M colony types from purified marrow progenitor cells.

ACKNOWLEDGMENT

We thank L.I. van Eijk for indispensable technical assistance and M. Drinkwaard for typing the manuscript.

REFERENCES

1. Metcalf D: The hemopoietic colony stimulating factors. Elsevier, Amsterdam, 1984

2. Wong GG, Witek JS, Temple PA, Wilheus KM, Leary AC, Luxenberg DP, Jones SS, Brown EL, Kay RM, Orr EC, Shoemaker C, Golde DW, Kaufman RJ, Hewich RM, Wang EA, Clark SC: Human GM-CSF: Molecular cloning of the cDNA and purification of the natural and recombinant proteins. Science 228:810, 1985

3. Burgess AW, Begley CG, Johnson GR, Lopez AF, Williamson DJ, Mermod JJ, Simpson RJ, Schmitz A, Delamartez J: Purification and properties of bacterially synthesized human granulocyte-macrophage colony stimulating factor. Blood 69:43, 1987

4. Nagata S, Tsuchiya M, Asano S, Kaziro Y, Yamazahi T, Yamamoto O, Hirata Y, Kubota N, Oheda M, Nomura H, Ono M: Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. Nature 319:415, 1986

5. Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, Clarin VR, Bruszewski J, Lu H, Cheu KK, Barendt J, Platzer E, Moore MAS, Mertelsmann R, Welte K: Recombinant human granulocyte colony stimulating factor: Effects on normal and leukemic myeloid cells. Science 232:61, 1986

6. Kawasaki ES, Ladner MB, Wang AM, Van Andell J, Warren MK, Coyne MY, Schweichart VL, Lee MT, Wilson KJ, Boosman A, Stanley ER, Ralph P, Mark DF: Molecular cloning of a complementary DNA encoding human macrophage-specific colony-stimulating factor (CSF-1). Science 230:292, 1985

7. Ihle JN, Keller J, Oroszlan S, Henderson LE, Copeland T, Fith F, Prystowsky MB, Goldwasser E, Schrader JW, Palaszynsky E, Dy M, Lebel B: Biologic properties of homogenous interleukin 3. J Immunol 131:282, 1983

8. Dorssers L, Burger H, Wagemaker G: Identity of murine stem cell activating factor (SAF) and interleukin-3 (IL-3) and common specificity for pluripotent stem cells. Exp Hematol 12:357, 1984

9. Hapel AJ, Fung MC, Johnson RM, Young IG, Johnson G, Metcalf D: Biologic properties of molecularly cloned and expressed murine interleukin-3. Blood 65:1453, 1985

10. Dorssers L, Burger H, Bot FJ, Delwel R, Geurts van Kessel AHM, Löwenberg B, Wagemaker G: Characterization of a human multilineage colony stimulating factor cDNA clone identified by a conserved noncoding sequence in mouse interleukin-3. Gene 55:115, 1987

11. Yang YC, Ciarletta AB, Temple PA, Chung MP, Kovacic S, Witek-Gianotti JA, Leary AC, Kriz R, Donahue RE, Wong GG, Clark SC: Human IL-3 (multi-CSF): Identification by expression cloning of a novel hematopoietic growth factor related to murine IL-3. Cell 47:3, 1986

12. Katz FE, Tindle R, Sutherland DR, Greaves MF: Identification of a membrane glycoprotein associated with haemopoietic progenitor cells. Leuk Res 9:191, 1985

13. Majdic O, Bettelheim P, Stockinger H, Aberer W, Liszka K, Lutz D, Knapp W: M2, a novel myelomonocytic cell surface antigen and its distribution on leukemic cells. Int J Cancer 33:617, 1984

14. Van der Reijden HJ, Van Rhenen DJ, Lansdorp PM, Van 't Veer MB, Langenhuijsen MMAC, Engelfriet CP, Von dem Borne

AEGKr: A comparison of surface marker analysis and FAB classification in acute myeloid leukemia. Blood 61:443, 1983

15. Perussia B, Trinchieri G, Lebman D, Jankiewicz J, Lange B, Rovera G: Monoclonal antibodies that detect differentiation surface antigens on human myelomonocytic cells. Blood 59:382, 1982

16. Fauser AA, Messner HA: Granuloerythropoietic colonies in human bone marrow, peripheral blood and cord blood. Blood 52:1243, 1978

17. Ash RC, Detrick RA, Zanjani ED: Studies of human pluripotential hemopoietic stem cells (CFU-GEMM) in vitro. Blood 58:309, 1981

18. Aye MT, Niho Y, Till JE, McCulloch EA: Studies of leukemic cell populations in culture. Blood 44:205, 1974

19. Motoyoshi K, Suda T, Kusumoto K, Tahaku F, Miura Y: Granulocyte-macrophage colony stimulating and binding activities of purified human urinary colony-stimulating factor to murine and human bone marrow cells. Blood 60:1378, 1982

20. Horiguchi J, Warren MK, Kufe D: Expression of the macrophage specific colony stimulating factor in human monocytes treated with granulocyte-macrophage colony stimulating factor. Blood 69:1259, 1987

21. Warren MK, Ralph P: Macrophage growth factor CSF-1 stimulates human monocyte production of interferon, tumor necrosis factor, and myeloid CSF. J Immunol 137:2281, 1986