Preferential Proliferation of Murine Colony-forming Units in Culture in a Chemically Defined Condition with a Macrophage Colony-stimulating Factor–negative Stromal Cell Clone

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Summary

The establishment of culture conditions that selectively support hematopoietic stem cells is an important goal of hematology. In this study, we investigated the possibility of using for this purpose a defined medium, mSFO2, which was developed for stromal cell–dependent bone marrow cultures. We found that a combination of epidermal growth factor (EGF), the OP9 stromal cell line, which lacks macrophage colony-stimulating factor, recombinant stem cell factor, and the chemically defined medium mSFO2 provides a microenvironment where c-Kit+Thy-1+/loMac-1+/loB220−TER119−commonβ+IL-2Rγ+gp130+ cells are selectively propagated from normal, unfractionated bone marrow cells. This cell population produced an in vitro colony at a very high efficiency (50%), whereas it has only limited proliferative ability in the irradiated recipient. Thus, the cells selected in this culture condition might represent colony-forming units in culture (CFU-c) with short-term reconstituting ability. Transferring this cell population into medium containing differentiation signals resulted in the rapid production of mature myelomonocytic and B cell lineages in vitro and in vivo. The fact that a similar culture condition was created by erb-B2–transduced OP9 in the absence of EGF indicated that EGF exerts its effect by acting on OP9 rather than directly on CFU-c. These results suggested that the balance between self-renewal and differentiation of CFU-c can be regulated by extracellular signals.

An important goal of experimental hematology is to manipulate the proliferation and differentiation of the normal hematopoietic stem cells. As an approach to this end, extensive efforts have been made to establish cultures where the growth of hematopoietic stem cells is maintained. The stromal cell–dependent, long-term bone marrow (BM)1 culture developed by Dexter and his colleagues (1) has been the most successful means of maintaining hematopoietic stem cells in vitro. Although the sustained self-renewal of the multipotent stem cells is supported in this system, concomitant terminal differentiation into the myelomonocytic cell lineage does occur. As a result of this differentiation in culture, a minor population of multipotent stem cells persists, and the production of hematopoietic cells eventually ceases because of the imbalance between self-renewal and differentiation of the stem cells. Thus, any method that alters this balance in favor for self-renewal might result in a culture where hematopoietic progenitors are spontaneously enriched. In fact, this has been attained in cultured embryonic stem cells using leukemia inhibitory factor as a molecular cue (2, 3).

One way to change the balance in favor of self-renewal is to block the intracellular machinery that drives the hematopoietic stem cell to differentiate. However, once the intracellular machinery for cell differentiation is artificially locked, it is difficult to release it physiologically. Alternatively, extensive attempts have been made to determine the growth factor combination that allows the proliferation of stem cells while arresting cell differentiation (4–13), as leukemia inhibitory factor does to the embryonic stem cells. Moreover, a number of untransformed stem cell lines, some of which have been claimed to give rise to the whole

1 Abbreviations used in this paper: bFGF, basic fibroblast growth factor; BM, bone marrow; CFU-c, CFU in culture; CFU-s, CFU in spleen; EGF, epidermal growth factor; Epo, erythropoietin; G-CSF, granulocyte CSF; M-CSF, macrophage CSF; SCF, stem cell factor.
range of hematopoietic cell lineage, have been described (14–16). However, those cell lines have not been generally applied for two main reasons. Most, if not all, of these stem cell lines tend to lose sensitivity to differentiation signals, though a small proportion of them may differentiate upon stimulation either in vitro or in vivo. Moreover, it is usually difficult to reproduce the culture conditions described in the original reports because of the presence of chemically undefined ingredients in the media.

In this study, we investigated ways to overcome this problem. We reduced the differentiation factors present in ordinal long-term BM culture using the defined culture medium mSFO2, which contains transferrin and insulin as the only protein components, as well as the macrophage CSF (M-CSF)–negative stromal cell clone OP9.

**Materials and Methods**

**Cell line OP9.** The stromal cell line OP9 was maintained in α-MEM (GIBCO BRL, Gaithersburg, MD) containing 20% FCS (lot No. 1M1250; BioWhittaker, Walkersville, MD), as reported (17). The subline OP9/erbB2 that can survive in serum-free media without external growth factors was generated by means of calcium phosphate transfection performed essentially as reported (18). Briefly, exponentially growing OP9 cells were seeded at a density of 5 × 10^6 cells on 10-cm tissue culture plates in α-MEM with 20% FCS 1 d before transfection. On the day of transfection, calcium phosphate DNA solution was added drop-wise into the medium, and the cells were incubated for 24 h in a 37°C, 5% CO2 incubator. The OP9 cells were transfected by cotransfection with the pSV2erbB2VE plasmid containing the constitutive active phosphorylation site of erbB2 (19–21) in the cytoplasmic domain (a gift from T. Yamamoto, Institute of Medical Science, University of Tokyo, Tokyo, Japan), as well as the pSV2bsr plasmid vector (Funakoshi Co., Tokyo, Japan) containing the resistance gene for blasticidin S hydrochloride (Funakoshi Co.).

BM cells harvested from normal C57BL/6 mice were suspended in RPMI 1640 medium (GIBCO BRL) with 10% FCS and passed through Sephadex G-10 (Pharmacia, Uppsala, Sweden) twice to deplete stromal cells and macrophages (22, 23). Stromal cell–depleted BM cells (10^3) were seeded onto OP9 stromal cells under various conditions: in 3 ml of RPMI 1640 medium containing 10% FCS and 0.1% BSA, or in 3 ml of mSFO2 supplemented with either recombinant (r) epidermal growth factor (EGF; 20 ng/ml; Peprotech Inc., Rocky Hill, NJ) or recombinant basic fibroblast growth factor (rbFGF; 10 ng/ml; GIBCO BRL) with or without recombinant stem cell factor (rSCF; 100 ng/ml; reference 24) on PRIMA1RA culture plates measuring 3.5 cm in diameter (Becton Dickinson Labware, Lincoln Park, NJ). The concentration of SCF was decided based on a previous study showed that the RPMI 1640/DMEM/F12-based defined medium mSFO2 (Sanko Junyaku, Chiba, Japan), which contains transferrin and insulin as the only protein components, supported hematopoiesis on the stromal cell layer was initiated and maintained in the same manner, except that EGF and bFGF were omitted from the medium.

**Antibody and Flow Cytometry.** The mAbs used in immunofluorescence staining for lineage markers were Mac-1 (M1/70), B220 (RA3-6B2), anti-CD4 (GK1.5), anti-CD8 (53-6.72), and TER119 (erythroid lineage marker; 25). THY1 (Thy1.2), ACK4 (anti-c-Kit; 26, 27), A7R (anti-IL-7 receptor; 28), ASF98 (anti-c-Fms; 29), GP130 (anti-gp130, a gift from Dr. T. Taga, Osaka University, Osaka, Japan; references 30–33), AIC2B (anti-common γ chain for IL-2, -4, and -7; a gift from Dr. K. Sugamura, Tohoku University, Sendai, Japan; references 36, 37) were also applied. All mAbs were purified and conjugated with FITC or biotin. Biotinylated antibodies were visualized with PE-conjugated streptavidin (GIBCO BRL) or FITC-conjugated streptavidin (Sigma Chemical Co., St. Louis, MO). The stained cells were analyzed by EPICS XL (Coulter Electronics, Hialeah, FL).

**In Vitro and In Vivo Colony Assay.** In vitro colony formation in methylcellulose–containing semisolid medium was assayed as described (27). Briefly, cultured or fresh BM cells were incubated in 1 ml of culture medium containing α-MEM (GIBCO BRL), 1.2% methylcellulose (Methocel A-4M; Muromachi Kagaku Kogyo, Tokyo, Japan), 30% FCS (lot No. 1M1137; BioWhittaker), 1% deionized BSA (Sigma Chemical Co.), 30 μM 2-ME, 200 U/ml IL-3, and 2 U/ml erythropoietin (Epo; Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). On the 7th d of culture, aggregates consisting of >40 cells were counted as a colony.

For CFU in spleen (CFU-s) assay, C57BL/6 mice were purchased from Japan SLC Inc. (Shizuoka, Japan) and were used as BM donors and recipients at 8–10 wk of age. Recipient mice were maintained on acidified water (pH 2.5) 1 wk before the irradiation (9.2 Gy) with a 60Co source, and donor cells were injected the next day. 8 or 12 d thereafter, the number of colonies that appeared in the recipient spleen was counted after fixation in Bouin’s solution (38, 39).

**Results**

**Distinct Types of Hematopoietic Cells Were Generated on OP9 Stromal Cells Stimulated by EGF or bFGF.** Our previous study showed that the RPMI 1640/DMEM/F12–based defined medium mSFO2 (Sanko Junyaku, Chiba, Japan), which contains transferrin and insulin as the only protein components, supported hematopoiesis on the stromal cell lines ST2 and PA6 (40). To reduce the number of factors present in this BM culture, we initially examined whether the stromal cell line OP9 (41), which was derived from the M-CSF–deficient op/op mouse (42), can be used for the BM culture with mSFO2 instead of ST2 or PA6, which both express M-CSF. Moreover, because 2-ME is essential for differentiation into B cell lineage, it was removed from the original culture. Although OP9 cells could not survive under serum deprivation, addition of either rEGF or rbFGF solved this problem. While these in vitro microenvironments supported the growth of fresh BM cells, proliferation remained at a low level compared with that of stromal cell–dependent hematopoiesis in the medium-containing serum (see Fig. 2 A and data not shown). To enhance the proliferation of hematopoietic cells, we
Figure 1. Phenotypes of BM cells cultured under various conditions. Unfractionated BM cells (10⁵) from C57BL/6 mouse were plated onto the OP9 stroma cells and cultured in mSFO2 containing 10% FCS (A), 10 ng/ml bFGF, and 100 ng/ml SCF (B), or 30 ng/ml EGF and 100 ng/ml SCF (C) in 60-mm culture dishes for 14 d. (D) Unfractionated BM cells (10⁵) were plated onto OP9 stromal cells transformed with the v-erbB2 gene (OP9/erbB2) in the mSFO2 plus SCF (100 ng/ml) for 21 d. Cultured BM cells were analyzed by May–Grunwald-Giemsa staining (left) and by flow cytometry (right) after staining with biotinylated anti–c-Kit (ACK4), FITC-conjugated Mac-1, and anti-B220 antibody. Biotinylated ACK4 was detected with streptavidin-PE. The numbers in each quadrant represent the percentages of gated bone marrow cells. Bar, 10 μm.
added the ligand for c-kit (SCF) in the culture. The improvement was striking, as the cells extensively proliferated. More marked was the finding that the cells proliferating in the culture with EGF plus SCF retained an immature morphology, having a large nucleus and azurophilic granules (Fig. 1B), while those in the culture with bFGF plus SCF were heterogeneous, containing mature and immature myeloid cells (Fig. 1B). In complete agreement with this morphology, flow cytometry of the same cells showed that 98% of the cultured cells were c-Kit<sup>+</sup> in the EGF-containing culture. More than 60% of the cells in the bFGF-containing culture were c-Kit<sup>+</sup> (Fig. 1B), while it contained more Mac-1<sup>high</sup> macrophages than the cultures with FCS (Fig. 1A).

**Cell Kinetics in the EGF-containing Culture.** The above results indicated that the OP9 stromal cell line with EGF and SCF in the mSFO2-defined medium would provide a culture condition that allows the preferential growth of a subset of hematopoietic stem cells. Next, we investigated how long this selective proliferation of the hematopoietic stem cells lasts under this culture condition. The expression of c-Kit was used as a marker of the immature hematopoietic cells. In this particular experiment, exponential cell proliferation generating > a 1,000-fold increase in the cell number continued up to 40 d (Fig. 2A). At the initial phase of culture, the number of cultured cells increased ~100-fold (Fig. 2A), and the proportion of c-Kit<sup>+</sup> cells increased to 80% in the 21-d culture (Fig. 2B). Since normal BM consists of 14% c-Kit<sup>+</sup> cells, their net increase over 21 d was ~600-fold. Thereafter, however, the proportion of c-Kit<sup>-</sup> cells started to increase concomitantly with a decrease in cell proliferation. These results suggested that this culture condition is indeed selective for the c-Kit<sup>+</sup> population during the initial 3 wk, but it cannot block differentiation over a longer period.

**EGF Acts on OP9 Stromal Cells to Produce the Microenvironment Selective for Hematopoietic Cells.** Beug et al. have demonstrated that TGF-α acts on chicken erythroid progenitors to maintain self-renewal without differentiation (43). This suggests that EGF, which binds to the same receptor as TGF-α (44), acts directly on murine hematopoietic progenitors to augment their self-renewal. However, we could not detect the direct binding of [125I]-labeled EGF to the cells proliferating under this condition (data not shown). An alternative is that EGF acts on OP9 stromal cells to create a microenvironment that is selective for the self-renewal of hematopoietic progenitors. To test this notion, we established an OP9 subline (OP9/erbB2) that was transduced with the v-erbB-2 gene containing a constitutive active phosphorylation site in the cytoplasmic domain. This cell line was maintained under serum-deprived conditions without exogenous growth factors.

Unfractionated BM cells (10<sup>5</sup>) were cultured on OP9/erbB2 in the presence of SCF, but without either EGF or bFGF, and the cells recovered from the culture were counted. As shown in Fig. 3A, cell growth was exponential under this culture condition, although the growth rate in this culture was slower than that in the culture with EGF. Importantly, the morphological features of the proliferating cells under this condition were similar to those cultured with EGF (Fig. 1D). This suggested that OP9 stromal cells, when properly stimulated, can create a microenvironment that is selective for c-Kit<sup>+</sup> Mac-1<sup>high</sup> cells in the absence of exogenous growth factors.

**Phenotype of the Cells Cultured with OP9/erbB2.** We analyzed the surface phenotype of the cells proliferating under this culture condition (OP9/erbB2 plus SCF). As shown in Fig. 3B, >80% of the culture consisted of c-Kit<sup>+</sup> immature cells. This c-Kit<sup>+</sup> population was positive for common β and common γ chain expression, dull to positive for Mac-1, Thy-1, and gp130 expression, and negative for c-FMS.

The clonogenic activity of this cell population was analyzed by means of in vitro colony-forming cell assays and by transfer into irradiated mice (Table 1). Consistent with the expression of the IL-3 receptor β-chain in this cell population, half of the cultured cells formed colonies in response to IL-3 plus Epo. Since the frequency of CFU in culture (CFU-c) in normal BM is ~1/140 (Table 1) and the cell number increased 100-fold during 21 d of culture (Fig. 3), the net increase of CFU-c was at least 7,000-fold. In contrast to this high in vitro colony-forming ability, the...
frequency of CFU-s measured at days 8 and 12 after transfer were 1/140 and 1/250, respectively. Thus, only a part of this cell population maintained CFU-s activity. Since frequencies of day 8 and day 12 CFU-s in normal BM cells were $\approx 1/5000$, their net increases were $\approx 3,600$- and $2,000$-fold, respectively. This indicates that while CFU-s could expand under this culture condition, the cells with long-term reconstituting activity were lost more rapidly than the more mature progenitors. These results suggested that the cells that were selected most preferentially under our culture conditions represent short-term reconstituting progenitors, probably CFU-c.

We next investigated whether this cell population, phenotypically representing CFU-c, preserves reactivity to differentiation signals. In fact, almost all colonies formed in response to IL-3+Epo contained mature myeloid and monocytic cells (data not shown), suggesting that most cells can readily differentiate. Since the culture with OP9 and medium-containing serum provides conditions that favor differentiation into mature hematopoietic cells (Fig. 1), we investigated whether or not this cell population would react to a shift from serum-deprived medium to medium containing serum and 2-ME. As shown in Fig. 4, there was a quick phenotypic shift, as the number of c-Kit$^+$ cells decreased, whereas the number of c-Kit$^+$Mac-1$^+$ cells increased to 45.8% 7 d after the shift. Thus, most cells maintained under our culture conditions were highly reactive to this shift and readily gave rise to mature myelomonocytic cells. At 14 d in culture, B220$^+$ cells were generated, suggesting that this cell population contained the progenitor cells for B cell lineage. A small proportion of B220$^+$ IL-7R$^+$ cells appeared before the B220$^+$ cells, which constituted

Table 1. Frequency of Colony-forming Cells

| Factor: IL-3 and Epo | Fresh BM per $5 \times 10^4$ cells | Cultured cells per $10^3$ cells | Control |
|---------------------|----------------------------------|---------------------------------|---------|
| No. of CFCs         | $372.5 \pm 13.2$                 | $523.0 \pm 34.0$                | 0*      |
| No. of CFU-S        | Day 8 11.2 $\pm$ 2.5 $\pm$ 2.5 | 7.0 $\pm$ 2.0 0                |
| Day 12 10.6 $\pm$ 2.6 | 4.0 $\pm$ 1.0 0.4 $\pm$ 0.6 |

*This colony number was obtained by counting fresh BM cells ($5 \times 10^4$ cultured in methylcellulose without IL-3 and Epo. The numbers in the control line indicate colony numbers of those of nuclear cell counts of BM in the control mice that were injected with PBS. The results represent the mean $\pm$ SE of triplicate samples. 

Figure 3. Growth kinetics of BM cells cultured on the OP9/erbB2 stromal layer and surface phenotype of recovered cells. (A) Unfractionated BM cells ($10^6$) derived from C57BL/6 were plated on OP9/erbB2 stroma cells in mSFO2 supplemented with 100 ng/ml SCF. On the indicated days, the cultured cells were harvested, counted, and replated onto the fresh OP9/erbB2 stromal layer. (B) Cells harvested on the 21st d of culture were analyzed by flow cytometry after staining with FITC-conjugated Mac-1, anti-B220, TER119, CD4, CD8, biotinylated anti-c-Kit (ACK4), Thy-1, anti–IL-7 receptor (A7R), anti–c–Fms (AFS98), anti-gp130 (GP130), anti–common $\beta$ (AIC2B), and anti–common $\gamma$ (TUGm3). Biotinylated antibodies were detected with streptavidin-PE. The vertical lines indicate the mean (left) and the maximal (right) fluorescence intensity of cells stained with an isotype-matched control mAb.

Figure 4. Immediate differentiation of cultured c-Kit$^+$Mac-1$^+$ cell in medium-containing serum. Unfractionated BM cells were cultured on OP9/erbB2, as described in the legend to Fig. 3. 21 d later, the cells were transferred onto a fresh OP9 stroma cell layer with medium-containing serum (RPMI 1640 with 10% FCS and $5 \times 10^{-3}$ M 2-ME). The recovered cells were analyzed after staining with FITC-conjugated Mac-1 and biotinylated anti-c-Kit (ACK4), or FITC-conjugated anti-B220 and biotinylated anti–IL-7 receptor (A7R) (A) before, (B) 7 d, or (C) 14 d after the transfer. Biotinylated antibody was detected with streptavidin-PE. The percentage of cells in each quadrant is shown in the top right-hand corner of each panel.
50% of the cells at the 14th d of culture. This suggested that IL-7 receptor expression precedes the expression of B220 during B lineage differentiation.

Discussion

Our results demonstrated that the proliferation of a subset of hematopoietic progenitors, most probably CFU-c, can be selectively supported in vitro, whereas that of more mature cells cannot. The optimal conditions for such selection were attained by using either the OP9 stromal cell line, SCF, and EGF, or by using v-erbB2–transduced OP9 cells and SCF. Cells that were c-Kit+ Mac-1dull did not selectively proliferate on the M-CSF–expressing stromal cell lines PA6 or ST2 (40). Moreover, medium-containing serum provided a microenvironment that favored hematopoietic differentiation, even if EGF was added to the culture (data not shown). The addition of 2-ME in mSFO2 promoted the proliferation of pre-B cells (c-Kit+ B220+ Mac-1−) at 21 d of culture, even in the presence EGF and SCF (data not shown). Therefore, the 2-ME–free, chemically defined medium and the M-CSF–deficient OP9 stromal cell line are essential components of these culture conditions. The addition of bFGF instead of EGF, however, produced mature hematopoietic cells. This suggested that an in vitro microenvironment formed by a uniform stromal cell line in the absence of serum could be varied according to the stimulation given to the stromal cells. Thus, the selective proliferation of c-Kit+ Mac-1dull cells described here appeared to be an outcome of a particular combination of stromal cell molecules expressed by EGF-stimulated or v-erbB2–transduced OP9. Nevertheless, these results indicated that hematopoiesis is regulated, not only by molecules directly acting on hematopoietic progenitors, but also by those that stimulate stromal cell components.

It is totally obscure why the stimulation of OP9 cells by EGF created a microenvironment that favored the self-renewal of immature hematopoietic progenitors, whereas bFGF could not. To examine whether more granulocyte colony stimulating factor (G-CSF) or GM-CSF is produced by OP9 stimulation with bFGF than with EGF, thereby generating mature hematopoietic cells, we tried to inhibit these signals in the culture with OP9 stimulated by bFGF. We inhibited G-CSF and GM-CSF signals, using G-CSF receptor protein fused with human IgG1 protein that saturates G-CSF (our unpublished data) and a neutralizing mAb against GM-CSF (19). Even though we simultaneously inhibited both G-CSF and GM-CSF signals, we could not block the generation of differentiated cells completely in culture containing bFGF (data not shown). Thus, it is very unlikely that the G-CSF or GM-CSF produced by OP9 is responsible for the difference in the microenvironment created by EGF or bFGF stimulation.

Nevertheless, in complete agreement with the description by Beug et al. (43) of chicken erythroid progenitors, our results suggest that the balance between the self-renewal and differentiation of nontransformed hematopoietic progenitors can be regulated by a combination of the molecules expressed in the microenvironment. This notion challenges the widely held view that differentiated hematopoietic progenitors, including committed progenitors, undergo only a limited number of cell divisions. We are currently investigating which molecules are induced specifically by EGF to support the self-renewal of c-Kit+ Mac-1dull progenitors.

The most important feature of our procedure for enriching CFU-c, as compared with others, lies in the fact that the hematopoietic progenitor cells spontaneously dominate the culture. This is all that is required to simply culture unfractionated BM cells under the conditions described here. However, stem cell clones cannot be established, because the dominance of hematopoietic progenitors does not last more than 30 d because of their eventual differentiation into mature cells that do not proliferate. It is unclear whether this limitation of our method in maintaining the dominance of the immature progenitors is caused by the limited life span of c-Kit+ Mac-1dull cells before terminal differentiation or the inability of our culture conditions to block their terminal differentiation. One line of evidence suggests that this cell population is highly sensitive to the differentiation cue. First, they are likely to represent short-term reconstituting progenitors rather than the most immature stem cells, because 50% of them formed in vitro colonies, whereas only 0.4% could form day 12 spleen colonies. Second, the expression of Mac-1 in this population agrees with the study of Heimfeld and Weissman (45, 46), who found that Mac-1 is expressed by short-term–reconstituted myeloid stem cells. Although our study and their cell-sorting study did not address the differential capacity of individual cells with this phenotype, Mac-1dull cells in our culture were by no means restricted to myeloid cell lineage, because they also gave rise to B lineage cells (Fig. 4). In fact, a recent study by Morrison et al. (47) has demonstrated that the Thy−1low Mac-1dull population generated cells of multiple lineages. Third, they are highly sensitive to the differentiation cue, because they promptly gave rise to mature blood cells both in vitro and in vivo. Thus, any changes of the environment in the culture that could be induced by stromal cell aging or even by an increase of hematopoietic cells themselves could alter the balance in favor of differentiation of the progenitors. To develop long-term stem cell cultures, it is important to determine how to avoid such changes in the culture conditions.

A number of attempts have been made to establish a stromal cell–dependent culture that selects hematopoietic progenitors. The reports of Dexter and his colleagues have shown that hematopoietic progenitors are spontaneously selected from unfractionated BM cells on a v-src–transduced stromal cell (48, 49). While this method is very similar to ours, it has not become popular, probably because the culture conditions are complex and include a high serum concentration, as well as undefined stromal cell components. In contrast, we used defined medium, only two recombinant growth factors, and a cloned stromal cell line. Palacios and Samaridis (50, 51) took the opposite approach...
and added a cocktail of growth factors and carefully selected FCS to a cultured stromal cell line to produce stem cell clones. Although they claimed to be able to establish progenitor clones, it has obvious problems in that (a) a large number of cloned cells (>5 × 10⁶) has to be transferred into the irradiated recipient to detect their differentiation; (b) they could not form in vitro colonies, which are important characteristics of normal hematopoietic progenitors; and (c) only sorted cells can be used to initiate the culture. Compared with the reported methods, ours is simple and reproducible, because it uses chemically defined conditions and a cloned stromal cell line. However, it is not effective for isolating cell clones. The c-Kit⁺ Mac-1⁻ cell population enriched in this culture readily differentiated in response to various molecular cues. More than half of it responded to IL-3 to form colonies that contained mature myelomonocytic cells. Moreover, when cultured on OP9 stromal cells together with FCS and 2-ME, they gave rise to both myeloid and B220⁺ B lymphocytes.

In conclusion, though we still need to use the stromal cell line of which the activity on the growth and differentiation of CFU-c has not been fully defined, we are the first to define the culture conditions that can enrich CFU-c. In view of increasing interest in the clinical application of the ex vivo expansion of hematopoietic stem cells, our results are encouraging because we have showed that self-renewal and differentiation of hematopoietic progenitors are somewhat controllable under defined culture conditions. Although we determined that the removal of M-CSF and serum was essential, while high levels of soluble SCF should be included in the culture condition, other conditions created by EGF-stimulated OP9 cells remain to be determined so that this technology can be applied to human BM culture.

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