

Megakaryocyte Growth and Development Factor Is a Potent Growth Factor for Primitive Hematopoietic Progenitors in the Human Fetus

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ABSTRACT

Megakaryocyte growth and development factor (MGDF), or thrombopoietin, has received considerable attention as a therapeutic agent for treating thrombocytopenia or for its use in the *ex vivo* culture of hematopoietic stem cells. MGDF is known to support the growth of a broad spectrum of hematopoietic precursors obtained from adult or neonatal tissues, but its effects on the growth of fetal progenitors and stem cells has not been studied. Human CD38⁺CD34²⁺ progenitors and CD38⁻CD34²⁺ cells, a population that contains stem cells, were isolated from midgestation liver and grown under defined conditions with MGDF and various cytokines known to support the growth of primitive hematopoietic precursors. In clonal assays of colony-forming cells (CFCs), MGDF supported the growth of 15–25% of candidate stem cells when combined with granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), flk-2/flt3 ligand, or stem cell factor. MGDF was observed to strongly support the early stages of hematopoiesis and expansion of high proliferative potential CFCs. More mature progenitors were expanded nearly 78-fold in 1 wk of culture with

MGDF+SCF+GM-CSF. MGDF alone was also found to support the short-term (2 d) survival of CD38⁻CD34²⁺ high proliferative potential CFCs. The effects of MGDF were more modest on CD38⁺CD34²⁺ progenitors with only additive increases in colony formation being observed. These findings suggest that MGDF administration in fetuses and neonates may strongly affect the growth and mobilization of primitive hematopoietic progenitors and that MGDF may find use in the *ex vivo* growth and expansion of fetal stem cells. (*Pediatr Res* **55**: 1050–1056, 2004)

Abbreviations

CFC, colony-forming cell
FL, flk-2/flt3 ligand
G-CSF, granulocyte colony-stimulating factor
GM-CSF, granulocyte-macrophage colony-stimulating factor
HPP, high proliferative potential
LPP, low proliferative potential
MGDF, megakaryocyte growth and development factor
SCF, stem cell factor

Megakaryocyte growth and development factor (MGDF) is the major regulator of megakaryocyte development and platelet production (reviewed in Refs. (1, 2)). MGDF has been isolated from the sera of a number of mammalian species, in which its levels have been found to correlate inversely with peripheral blood platelet counts. Furthermore, the administration of MGDF has shown this cytokine to be a potent stimulator of thrombopoiesis, hence its other name thrombopoietin. The thrombopoietic effects of MGDF are the result of its ability to promote the growth and maturation of megakaryocytes. MGDF

can stimulate, both *in vitro* and *in vivo*, an increase in megakaryocyte production and megakaryocyte ploidy. Administration of MGDF as a treatment for neonatal thrombocytopenia is being studied (3–5).

MGDF also supports the growth of multipotent hematopoietic progenitors and stem cells. The administration of MGDF to mice after cytoablative injury has resulted in an accelerated recovery of myeloid and multilineage progenitors in addition to an increase in megakaryocytic progenitors (6). Moreover, mice deficient in MGDF or its receptor, *c-mpl*, have reduced numbers of committed and multipotent progenitors (7, 8), indicating a role for MGDF in the *in vivo* regulation of the early stages of hematopoiesis. These findings are further supported by observations of synergism between MGDF and other cytokines in increasing the growth of purified stem cells isolated from murine adult bone marrow (9, 10). In humans, *c-mpl* mRNA expression was detected among adult bone marrow cells with the phenotypic characteristics (CD38⁻CD34²⁺) associated with stem cells (11). MGDF alone

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was shown to stimulate the production of megakaryocytes and neutrophils from CD38⁻CD34²⁺ cells grown under serum-deprived conditions (12). MGDF was also observed to synergize with a number of other early-acting growth factors to promote the growth of human primitive progenitors (13, 14). MGDF has further found application in protocols used to expand hematopoietic progenitors *ex vivo* owing to the broad range of hematopoiesis supported by MGDF (2, 15).

The primary site of MGDF production throughout human ontogeny is the liver (16–18). MGDF mRNA has also been detected in the fetal and adult bone marrow as well as in organs such as kidney and spleen. The levels of MGDF in plasma are higher in the fetus and neonate than in the adult (17, 19, 20). The presence of MGDF in fetal hematopoietic tissues, such as the liver and bone marrow, indicates that this cytokine is a likely physiologic regulator of fetal hematopoiesis. Megakaryocyte progenitors isolated from human fetal tissues have been shown to respond to MGDF *in vitro* (21–24). MGDF has also been found to support the growth, when combined with either IL-3 or stem cell factor (SCF), of primitive progenitors isolated from murine fetal livers (25). The response of fetal hematopoietic progenitors to cytokines has been found to differ from that of adult and even neonatal progenitors. Fetal progenitors generate a larger number of progeny and can differ in their response to cytokines compared with their adult counterparts (26–31). The role of MGDF in the regulation of the early stages of fetal hematopoiesis in the human is unclear. The goal of this study was to determine whether MGDF can support the growth of primitive hematopoietic progenitors isolated from human fetal liver because MGDF may have application in the realm of fetal and neonatal therapies.

METHODS

Isolation of human fetal liver progenitors. Human fetal livers were obtained from elective abortions at the University of California San Francisco and from Advanced Bioscience Resources (Alameda, CA, U.S.A.). Fetal tissue was used with the approval of the Committee for Human Research at University of California San Francisco and with the consent of the women undergoing the abortion. The gestational age of the tissues was estimated on the basis of the foot-length of the fetus and ranged from 16 to 24 wk. A detailed protocol describing the isolation of lineage-depleted (CD3⁻, CD14⁻, CD19⁻, CD20⁻, and CD56⁻) CD38⁺CD34²⁺ and CD38⁻CD34²⁺ fetal liver cells used in this study has been published and is freely available on the internet (32).

Cytokines. Recombinant human MGDF, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), flk-2/flt3 ligand (FL), and SCF were provided by Amgen (Thousand Oaks, CA, U.S.A.). MGDF, G-CSF, and GM-CSF were used at 20 ng/mL unless otherwise stated. FL and SCF were used at 100 ng/mL and 50 ng/mL, respectively. These cytokine concentrations support the growth of a maximal number of colony-forming cells (CFCs) in assays performed under serum-deprived culture conditions.

CFC assays. Myeloid CFC numbers were determined in triplicate double-layered clonal cultures as previously described

(29, 30). These cultures consisted of a total volume of 3 mL of serum-deprived culture medium (33) consisting of IMDM (Life Technologies, Grand Island, NY, U.S.A.) supplemented with 7.5×10^{-5} M α -thioglycerol (Sigma Chemical Co., St. Louis, MO, U.S.A.), 50 μ g/mL gentamicin, 2% fraction-V ethanol-extracted BSA (Roche Applied Science, Indianapolis, IN, U.S.A.), 200 μ g/mL human iron-saturated transferrin (Serologicals Proteins, Kankakee, IL, U.S.A.), 10 μ g/mL rh-insulin (Roche Applied Science), and 20 μ g protein/mL human LDL (Sigma Chemical Co.). SeaPlaque agarose (FMC, Rockland, ME, U.S.A.) was used as the semisolid matrix for these cultures at a concentration of 0.5% in the lower layer and 0.36% in the upper layer. After 3 wk of growth at 37°C in a fully humidified atmosphere, three classes of CFC were scored on the basis of the size of the hematopoietic colonies generated: high-proliferative potential (HPP) CFCs, low-proliferative potential (LPP) CFCs, and clusters. HPP-CFCs were defined by colonies that contained approximately 1×10^4 to 1×10^6 cells (29, 34). LPP-CFCs were defined by colonies smaller than HPP-CFCs but >50 cells/colony. Clusters were defined as colonies that contained 10–50 cells. The sum of all three CFC types is referred to as total CFCs. Multipotent CFCs that are capable of generating erythroid and myeloid cells were detected in methyl cellulose-based cultures as previously described in detail (35).

Delayed-cytokine-addition experiments were used to study the effects of MGDF on the survival of CD38⁻CD34²⁺Lin⁻ fetal liver progenitors as previously reported (30, 31). Briefly, clonal cultures were established using the serum-deprived culture medium with or without MGDF. A second addition of cytokines was performed at 1, 2, 3, 4, or 7 d after the initiation of culture, resulting in a final growth stimulus of MGDF+SCF+GM-CSF. Cytokines added after the initiation of the cultures were added in a volume of 30 μ L to the top of the semisolid cultures. Cultures stimulated with MGDF+SCF+GM-CSF from the beginning of culture were used to determine the number of CFCs seeded. SCF and GM-CSF were used in these cultures because these cytokines stimulate a broad range of myeloid progenitors and support the growth of a high frequency of HPP-CFCs (30).

Delta assay. The effects of MGDF, alone or in combination with other cytokines, on the growth of primitive progenitors during liquid culture were determined using the Δ assay (30). Duplicate or quadruplicate liquid cultures were initiated with 900 CD38⁻CD34²⁺ fetal liver cells in 1 mL of serum-deprived medium supplemented with or without cytokines. Primary CFCs were determined in parallel from triplicate cultures of 300 CD38⁻CD34²⁺Lin⁻ fetal liver cells grown in serum-deprived medium supplemented with SCF+GM-CSF. After 7 d of liquid culture, cells were harvested and washed, and secondary clonal cultures were established using identical culture conditions as used for the primary assays. The secondary CFC assays were initiated with dilutions of the cultured cells. The dilutions were determined on the basis of the degree of cellular expansion observed with the different cytokine combinations and were made to seed ~750 cells/culture when a sufficient degree of expansion had occurred. The change (Δ) in the number of cells and CFCs between the onset of liquid

culture and after 7 d of growth was determined by dividing the primary by the secondary values.

Data presentation and statistical analysis. The data are presented as the mean \pm 1 SEM of the results obtained from multiple experiments. The results of replicate CFC measurements for each individual experiment were averaged before use in calculating the mean of multiple experiments. The unpaired *t* test was used to determine the probability of differences between culture conditions from individual experiments. Differences were considered significant at $p \leq 0.05$.

RESULTS

MGDF supports colony formation by candidate stem cells.

For establishing that MGDF supports the growth of primitive progenitors from human fetal liver, purified progenitors were grown in clonal assays under serum-deprived conditions (Fig. 1). Two precursor populations were studied, $CD38^-CD34^{2+}Lin^-$ and $CD38^+CD34^{2+}Lin^-$ cells. The former population, lacking CD38 expression, contains stem cells, although it may also contain committed progenitors. The latter population, which expresses CD38, is composed of immature progenitors that do not have stem cell capacity (29, 32, 35).

MGDF alone supported colony formation by a small number of $CD38^-CD34^{2+}Lin^-$ candidate stem cells (Fig. 1). An average of 6 ± 2 total CFCs/ 1.0×10^3 $CD38^-CD34^{2+}Lin^-$ cells were detected in cultures with MGDF, whereas no colony formation was detected in cultures in the absence of any added growth factors ($p \leq 0.014$, $n = 3$). The effects of MGDF were compared with those of G-CSF, GM-CSF, FL, and SCF, growth factors that have been shown previously to support the growth of $CD38^-CD34^{2+}Lin^-$ fetal liver cells (30, 31). MGDF supported the growth of a greater number of total CFCs in two of three experiments compared with G-CSF and GM-

CSF ($p < 0.05$). The numbers of CFCs supported by MGDF and FL were similar, whereas SCF supported the growth of more total CFCs than observed with MGDF, G-CSF, GM-CSF, and FL ($p < 0.044$, $n = 3$). The level of proliferation supported by any of these cytokines individually was very low, with most colonies containing < 50 cells.

Synergism in supporting colony formation was observed between MGDF and G-CSF, GM-CSF, FL, or SCF (Fig. 1). Combining MGDF with a second cytokine increased colony formation from 3.7-fold with MGDF+FL to 5.5-fold with MGDF+SCF. Approximately one quarter of all $CD38^-CD34^{2+}Lin^-$ cells placed in culture formed colonies in the presence of MGDF+SCF. In addition to recruiting more progenitors to form colonies, combining MGDF with a second cytokine resulted in a modest increase in colony size. This was apparent by the presence of colonies derived from LPP-CFCs in the cultures supported by two cytokines, although no HPP-CFCs were detected under any of the conditions tested. These findings indicate that MGDF directly supports the growth of $CD38^-CD34^{2+}Lin^-$ cells originating from fetal liver.

MGDF supports the survival of HPP-CFCs. $CD38^-CD34^{2+}Lin^-$ fetal liver cells are enriched for progenitors with an extensive *in vitro* proliferative capacity, consistent with the presence of stem cells among this population of cells (29). Because none of these HPP-CFCs were detected in cultures of $CD38^-CD34^{2+}Lin^-$ cells grown under any of the culture conditions shown in Fig. 1, the possibility that MGDF affected the growth only of a more mature subpopulation of these cells existed. This possibility was disproved in experiments in which clonal cultures of $CD38^-CD34^{2+}Lin^-$ cells were initiated with or without MGDF, followed by a second addition of growth factors that supported the growth of HPP-CFCs. The combination SCF+GM-CSF was used in these

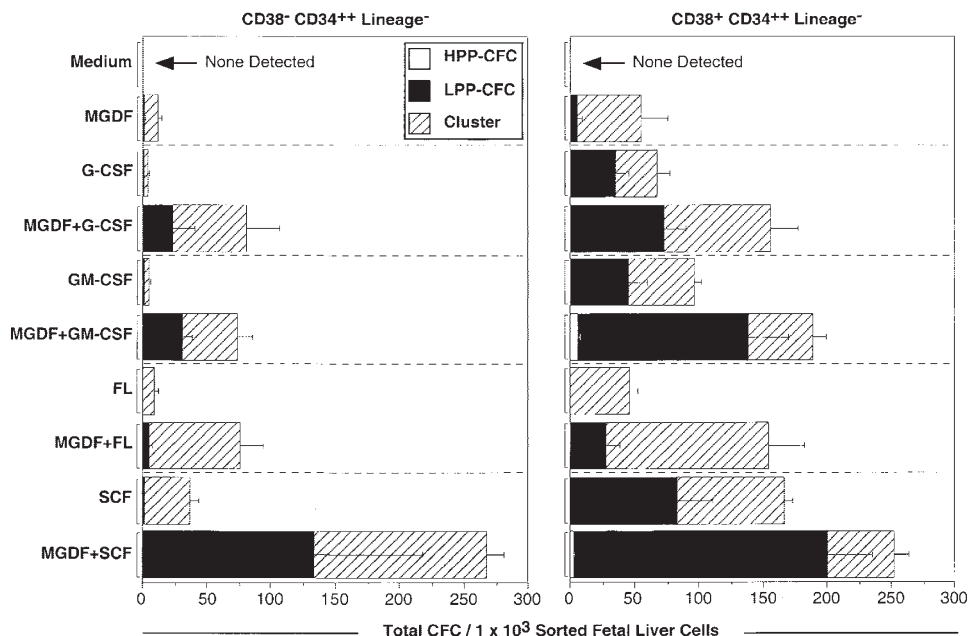


Figure 1. Effects of MGDF on colony formation by $CD38^-CD34^{2+}Lin^-$ and $CD38^+CD34^{2+}Lin^-$ cells. The mean \pm SEM of CFCs—segregated on the basis of size into clusters, LPP-CFCs, and HPP-CFCs—are indicated per 1×10^3 sorted fetal liver cells. Three experiments were performed using $CD38^-CD34^{2+}Lin^-$ cells, and four experiments were performed using $CD38^+CD34^{2+}Lin^-$ cells.

experiments to support the growth of HPP-CFCs (30). As shown in Fig. 2A, the inclusion of MGDF in cultures stimulated by SCF+GM-CSF did not significantly change the number of HPP-CFCs or LPP-CFCs detected ($p > 0.22$, $n = 3$).

Delaying the addition of growth factors by at least 2 d resulted in a significant decline in CFC viability in cultures initiated in the absence of cytokines (Fig. 2B). In contrast, both HPP-CFCs and LPP-CFCs were better maintained by the

presence of MGDF at the initiation of culture. CFCs did show a time-dependent decrease in cultures initiated with MGDF. The decreases were modest and usually insignificant during the first 3 d of culture. By the fourth day of culture, the numbers of CFCs detected in either cultures initiated with MGDF or those begun in the absence of cytokine had declined significantly. However, increased survival of CFCs cultured in the presence of MGDF was observed even when the addition of

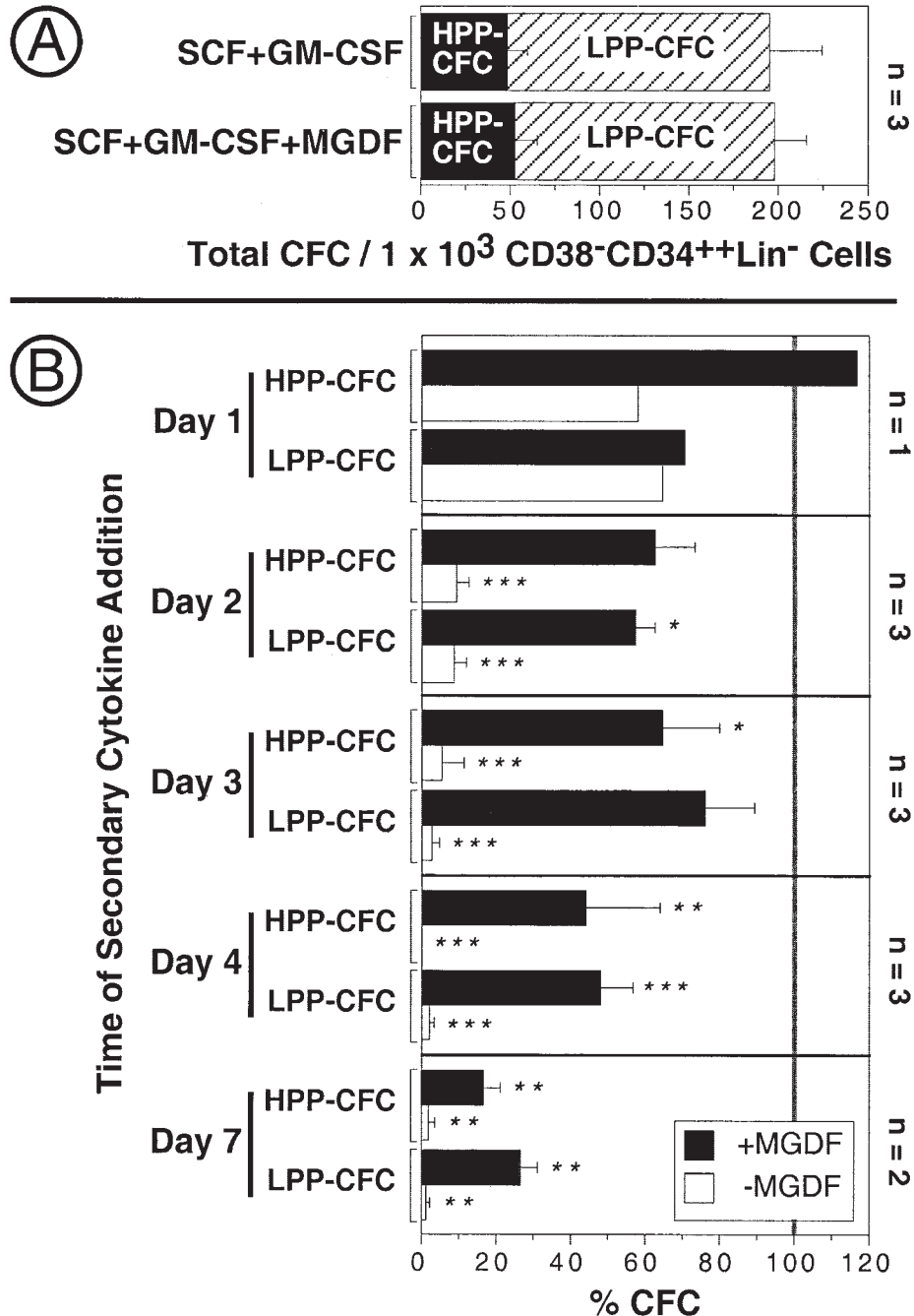


Figure 2. Effects of MGDF on the survival of CD38⁻CD34⁺Lin⁻ progenitors. (A) The cytokine combination SCF+GM-CSF was used to support the growth of a high frequency of HPP-CFCs and LPP-CFCs. The inclusion of MGDF in the combination of SCF+GM-CSF did not affect colony formation. (B) Cultures were initiated in the absence of cytokine or in the presence of MGDF. At 1 to 7 d of culture, cytokines were added such that each culture contained SCF+GM-CSF+MGDF. The data are presented as the percentage of HPP-CFCs and LPP-CFCs detected in cultures stimulated by SCF+GM-CSF+MGDF at the onset of culture. The results are shown as the mean \pm SEM of the number (n) of experiments indicated. *Significant differences between the number of HPP-CFCs and LPP-CFCs detected at the onset of culture and those detected in cultures to which cytokine addition was delayed. The numbers of asterisks denote the number of experiments in which the differences were found to be significant.

SCF+GM-CSF was delayed until 7 d of culture. These data demonstrate that MGDF directly affects at least a subset of $CD38^-CD34^{2+}Lin^-$ progenitors in the absence of any other growth factors and that some of the progenitors responsive to MGDF have the potential for a high degree of proliferation.

MGDF supports colony formation by immature progenitors. MGDF also supported significant levels of colony formation by $CD38^+CD34^{2+}Lin^-$ ($p \leq 0.013$, $n = 4$ experiments; Fig. 1). In a dose-response experiment, the number of colonies supported by MGDF reached a peak at 20 ng/mL, but significant growth was observed even at 0.5 ng/mL (data not shown), indicating that the 20 ng/mL of MGDF used throughout this study was optimal. The numbers of total CFCs supported by MGDF was comparable to that supported by either G-CSF or FL but was somewhat less than that supported by GM-CSF ($p \leq 0.016$, $n = 3$ of 4 experiments). SCF consistently supported more CFC growth than MGDF ($p < 0.001$, $n = 4$ experiments).

Combining MGDF with any of the other cytokines tested resulted in increased growth of $CD38^+CD34^{2+}Lin^-$ progenitors, similar to what was observed with the cultures of candidate stem cells. However, the degree of synergism observed with the cytokine combinations was low, ranging from 1.1- to 1.5-fold, compared with the super-additive effects on the more primitive $CD38^-CD34^{2+}Lin^-$ precursors. The numbers of total CFCs detected in cultures stimulated with two cytokines was significantly higher than the numbers detected with the individual cytokines in all but one sample ($p \leq 0.05$, $n = 4$).

Synergism between MGDF and other growth factors in promoting the expansion of progenitors. The effects of MGDF on the growth of $CD38^-CD34^{2+}Lin^-$ cells were further assessed using the Δ assay (Fig. 3). In seven experiments, the number of HPP-CFCs recovered from MGDF-stimulated cultures was 1.3 ± 0.4 -fold higher than input, which was comparable to the recovery of HPP-CFCs from SCF-stimulated cultures ($p > 0.05$). In the majority of experiments, the maintenance of HPP-CFCs by

MGDF was significantly greater than that supported by G-CSF, GM-CSF, or FL ($p \leq 0.05$). The results were similar for LPP-CFCs. The pattern observed using the Δ assay mirrored the pattern of colony growth from $CD38^-CD34^{2+}Lin^-$ progenitors supported by individual growth factors (Fig. 1). The Δ assay data further indicate that MGDF does promote the growth of a subset of early progenitors with a high level of proliferative potential even though HPP-CFCs were not detected in clonal cultures supported by MGDF alone.

Notable expansion of CFCs required combinations of cytokines (Fig. 3). The synergism between MGDF and SCF resulted in the greatest average expansion of HPP-CFCs ($\Delta 10.7 \pm 2.9$) and LPP-CFCs ($\Delta 26.8 \pm 6.9$) observed with any of the cytokine pairs that contained MGDF ($n = 7$). However, the expansion of HPP-CFCs supported by MGDF+SCF was less than half the 22.5 ± 8.1 -fold expansion stimulated by SCF+GM-CSF, although the differences between these two groups were significant in only three of seven experiments. Thus, the combination MGDF+SCF likely promotes the growth of the same population of HPP-CFCs as SCF+GM-CSF, because combining MGDF with SCF+GM-CSF promoted a similar expansion of HPP-CFCs as seen with SCF+GM-CSF. The combination MGDF+SCF+GM-CSF did provide the greatest expansion of LPP-CFCs ($\Delta 77.8 \pm 15.9$), which was greater than the combined effects of MGDF+SCF and SCF+GM-CSF ($p < 0.05$ in at least six of seven experiments). These results indicate that the triadic cytokine combination supports the growth of a broader spectrum of progenitors that develop during the 1-wk culture period than do MGDF+SCF or SCF+GM-CSF.

DISCUSSION

Hematopoiesis is regulated by a variety of growth factors that act at different stages of hematopoiesis to support or inhibit

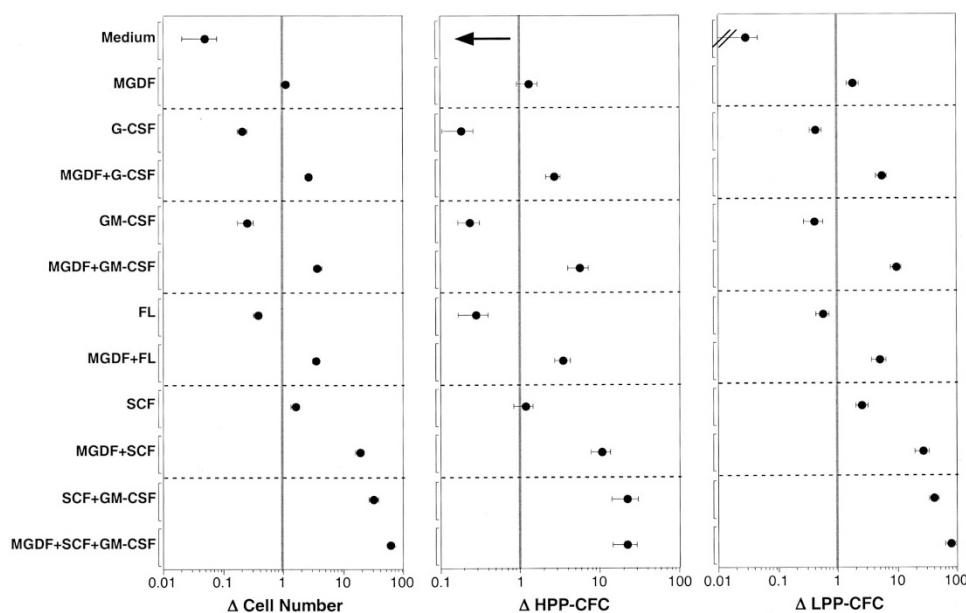


Figure 3. Effects of MGDF on the expansion of myeloid progenitors from $CD38^-CD34^{2+}Lin^-$ cells. The data are presented as the mean change (Δ) in cell, HPP-CFC, and LPP-CFC numbers \pm SEM ($n = 7$ to 8 experiments). Δ HPP-CFC could not be calculated for cells grown in serum-deprived medium alone (arrow), because no secondary HPP-CFCs were detected. Note that the Δ values are shown on a logarithmic scale and that vertical lines mark the input values.

the production of specific lineages of blood cells. The production of different cell lineages is in many cases regulated primarily by a single growth factor, such as the control of erythropoiesis by erythropoietin or support of megakaryocytopoiesis and thrombopoiesis by MGDF. In contrast, the early events in hematopoiesis are susceptible to regulation by a large number of cytokines, many of which are also important regulators of the final stages of hematopoietic development for specific cell lineages. MGDF is among these pleiotropic cytokines in that it has a broad spectrum of activity on hematopoiesis (1, 2). Herein, we demonstrate that MGDF is an important regulator of the growth of early hematopoietic progenitors and, likely, stem cells in the human fetus.

Several lines of evidence indicate that MGDF is capable of supporting the growth and survival of fetal primitive progenitors and/or stem cells. MGDF was found to support the growth of CFCs found among $CD38^-CD34^{2+}Lin^-$ cells. These candidate stem cells are known to require the presence of at least two cytokines for more than just a small number of CFCs to grow (30, 31). Indeed, synergism was observed between MGDF and each of the other early-acting cytokines tested. Synergism in stimulating the growth of $CD38^-CD34^{2+}Lin^-$ cells was also observed in the Δ assay, which demonstrated that MGDF could support the survival and expansion of CFCs. The size of colonies generated in the presence of MGDF and the degree of progenitor expansion supported by combinations of cytokines including MGDF was lower than with combinations such as SCF+GM-CSF. This observation is attributed to the limited range of hematopoietic activity that MGDF has on the late stages of hematopoiesis and the relatively few numbers of megakaryocytes generated as a result of endomitosis. Although MGDF did not produce large colonies when paired with other early-acting cytokines, the results from delayed-cytokine-addition experiments and from the Δ assay demonstrated that MGDF did support the growth of HPP-CFCs. Indeed, the ability of MGDF alone to support the short-term survival of HPP-CFCs observed in the delayed-cytokine-addition experiments was similar to that previously observed of SCF, FL, GM-CSF, G-CSF, and IL-3 on fetal $CD38^-CD34^{2+}Lin^-$ cells (30, 31). Because MGDF did not enhance the growth of $CD38^-CD34^{2+}Lin^-$ cells observed with SCF+GM-CSF, it can be deduced that MGDF supports the growth of the same progenitors as affected by SCF and GM-CSF. Together these findings indicate that MGDF is active in supporting the growth of $CD38^-CD34^{2+}Lin^-$ cells and can be included with SCF, FL, GM-CSF, G-CSF, and IL-3 in the category of fetal stem cell growth factors (30, 31).

As with most tissues in the fetus, hematopoietic tissues undergo a level of growth unparalleled later in life to accommodate the need both to expand the size of the hematopoietic tissues and to keep pace with the increasing volume of peripheral blood. A number of studies have indicated differences in how fetal and postnatal progenitors respond to growth factors and growth inhibitors (27–30, 36). Fetal stem cells are also known to have a greater proliferative capacity both *in vitro* and *in vivo* (26, 37). The findings in this study reveal further differences in how fetal and adult hematopoietic progenitors respond to cytokines. Although primitive progenitors/stem

cells from both fetal and adult sources respond to MGDF, the response of fetal tissues seems to be greater. In a study by Kobayashi *et al.* (13), MGDF was not found to stimulate any proliferation of adult candidate stem cells when grown in single-cell/well cultures in contrast to our observation that MGDF, alone, could support the modest expansion of CFCs from a small fraction of $CD38^-CD34^{2+}Lin^-$ cells. Moreover, Kobayashi *et al.* (38) further demonstrated synergism among MGDF, SCF, and FL in supporting the growth of candidate stem cells isolated from adult bone marrow. In comparison, MGDF was relatively more potent in supporting the growth of fetal $CD38^-CD34^{2+}Lin^-$ cells. Notably, the synergism between MGDF and SCF in supporting the growth of CFC among these candidate stem cells was as effective as SCF+GM-CSF (30). These findings contribute to previous observations that fetal progenitors/stem cells have a lower requirement for cytokines to support their growth (27).

There is considerable interest in MGDF for its use in expanding transplantable hematopoietic cells *ex vivo* because of its early-acting properties as well as its potent thrombopoietic properties (2, 15). Neonatal or fetal grafts are likely to benefit the most from *ex vivo* expansion because the numbers of progenitors available from these tissues are often too small for large adult recipients. The numbers of hematopoietic progenitors that can be obtained from midgestation fetal tissues exceeds that found in umbilical cord blood, and *ex vivo* expansion could further increase these numbers (33, 39). Optimal conditions for the *ex vivo* expansion of progenitors need to be determined independently for fetal, neonatal, and adult tissues owing to the changes in the growth properties of hematopoietic precursors as they age. Nonetheless, optimal expansion depends on the presence of multiple growth factors that can support progenitors of different lineages as they develop from multipotent progenitors. Even though the number of fetal $CD38^-CD34^{2+}Lin^-$ cells supported by SCF+GM-CSF was the same as SCF+GM-CSF+MGDF, the latter combination supported a greater expansion of progenitors. This was likely due to MGDF's supporting the growth of $CD38^+CD34^{2+}Lin^-$ cells that developed during the course of the 7-d culture period. MGDF did support CFC growth from $CD38^+CD34^{2+}Lin^-$ cells, but there was no notable synergism between MGDF and the other cytokines examined. These data suggest that the progenitors found among the $CD38^+CD34^{2+}Lin^-$ cells that are responsive to MGDF differ from those affected by G-CSF, GM-CSF, FL, or SCF. This explanation accounts for the additive effects of these cytokines in supporting the growth of $CD38^+CD34^{2+}Lin^-$ cells and the greater expansion of progenitors observed with SCF+GM-CSF+MGDF.

The strong growth-promoting properties of MGDF on primitive hematopoietic progenitors from the fetus suggests that this cytokine may have use in fetal or neonatal therapy. MGDF may find use as a treatment for newborn thrombocytopenia and has been shown to be effective in increasing platelet counts in neonatal rhesus monkeys (3, 4). Administration of MGDF has also been shown to increase the mobilization of progenitors and stem cells (40–42). Gene therapy performed on hematopoietic stem cells isolated from fetal and/or neonatal blood is being studied as a means to treat a number of inherited disorders of the hematoi-

etic system (43–47). Mobilization of stem cells by MGDF administration may increase the yield of stem cells that can be harvested for therapy and returned to the fetal or neonatal circulation.

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