Mast Cell Growth Factor (*c-kit* ligand) Supports the Growth of Human Multipotential Progenitor Cells With a High Replating Potential

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The replating capability of human multipotential (colony-forming unit-granulocyte-erythrocyte-macrophage-megakaryocyte [CFU-GEMM]) and erythroid (burst-forming unit-erythroid [BFU-E]) progenitors was assessed in vitro as a potential measure of self-renewal using purified, recombinant (r) human (hu) or murine (mu) mast cell growth factor (MGF), a ligand for the *c-kit* proto-oncogene receptor. Primary cultures of human umbilical cord blood or adult human bone marrow cells were initiated in methylcellulose with erythropoietin (Epo) alone or in combination with rhu interleukin-3 (IL-3) or MGF. Individual day 14 to 18 CFU-GEMM or BFU-E colonies were removed from primary cultures and reseeded into secondary methylcellulose cultures containing a combination of Epo, MGF, and rhu granulocyte-macrophage colony-stimulating factor (GM-CSF). The data showed a high replating efficiency of cord blood and bone marrow CFU-GEMM in response to Epo + MGF in terms of the percentage of colonies that could be replated and the number of secondary colonies formed per replated primary colony. The average number of hematopoietic colonies and clusters apparent from replated cultures of cord blood or bone marrow CFU-GEMM stimulated by Epo + MGF was greater than with Epo + rhuIL-3 or Epo alone. Replated cord blood CFU-GEMM gave rise to CFU-GEMM, BFU-E, and GM colony-forming units (CFU-GM) in secondary cultures. Replated bone marrow CFU-GEMM gave rise mainly to CFU-GM in secondary cultures. A more limited capacity for replating of cord blood and bone marrow BFU-E was observed. These studies show that CFU-GEMM responding to MGF have an enhanced replating potential, which may be promoted by MGF. These studies also support the concept that MGF acts on more primitive progenitors than IL-3.

MATURE BLOOD CELLS are derived through the sequential differentiation of hematopoietic stem cells to progenitor cells, which divide, in turn, to produce more differentiated precursor cells. Self-renewal, the potential of a stem cell to duplicate itself, is a very important but poorly understood event in hematopoiesis.[1] The in vivo murine colony-forming unit-spleen (CFU-S) assay has been used to measure both stem cell self-renewal capacity and multipotential stem cell content,[2,3] although the CFU-S assay does not appear to recognize the marrow repopulating cell.[4] The in vitro counterpart of the murine CFU-S assay appears to be the murine stem (S) cell assay, in which the ability of single S-cell colonies to be replated into secondary cultures with resultant multipotential and lineage-restricted colony formation is a measure of S-cell self-renewal capacity.[5,6] While the S cell is considered to be the predecessor of the colony-forming unit-granulocyte-erythrocyte-macrophage-megakaryocyte (CFU-GEMM), the exact relationship of S cells to the CFU-S has yet to be exactly elucidated. The S-cell assay has been modified for the study of human hematopoietic cell populations.[7-11] Murine, and to a lesser extent human, S cells have "self-renewal" capability based on colony replating assays,[5-11] but CFU-GEMM, burst-forming unit-erythroid (BFU-E), and CFU-granulocyte-macrophage (CFU-GM) progenitors appear to have little or no ability to replate.[12-14]

The proliferation and differentiation of hematopoietic progenitors is regulated by a complex network of cytokines that are produced by accessory cells and those of the hematopoietic microenvironment.[15] The possibility exists that in early studies, culture conditions may not have been appropriate for the detection of progenitor cell replating due to the lack of a specific cytokine or cytokines. Recently, several groups have described soluble forms of the ligand for the product of the *c-kit* proto-oncogene,[16-23] a tyrosine kinase receptor[24] encoded by the white-spotting (W) locus on murine chromosome 5.[25,26] The ligand has been termed mast cell growth factor (MGF),[16-18] stem cell factor,[19-21] and kit ligand.[22,23] Membrane-bound forms of the ligand,[18,27] which is the product of the murine steel locus,[17,21,23] have also been observed. MGF is an early acting hematopoietic growth factor that stimulates macroscopic BFU-E and CFU-GEMM colonies when used in combination with erythropoietin (Epo) in vitro.[16,19,28-30] Both the human and murine forms of MGF when used at optimal concentrations have equally potent costimulatory activity in the presence of colony-stimulating factors for human bone marrow hematopoietic progenitor cells.[30] We wanted to investigate the effects, if any, of MGF on the replating potential of human hematopoietic progenitor cells.

Based on assessments of hematopoietic progenitor cells in single collections of human umbilical cord and placental blood, these cell populations have been described as potential sources of stem/progenitor cells for hematopoietic reconstitution.[31] Because successful hematopoietic engraftment has been observed in Fanconi anemia[32,33] and juvenile chronic myelogenous leukemia[34] using umbilical cord blood from HLA-identical siblings, we wanted to determine the replating capacity of progenitor cells present in this cell population. The replating capabilities of human umbilical cord blood CFU-GEMM and BFU-E that formed

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colonies in the presence of Epo plus purified rhu or recombinant human (rhu) or recombinant murine (rmu) MGF was evaluated. This capability was compared with the replating potential of human adult bone marrow CFU-GEMM and BFU-E using single-colony transfer assays in vitro.

MATERIALS AND METHODS

Cells and cell separation procedures. Bone marrow cells were obtained by aspiration from the posterior iliac crest of healthy volunteers who had given informed consent according to the guidelines established by the Human Investigation Committee of the Indiana University School of Medicine (Indianapolis). Ficoll/Hypaque (Pharmacia LKB Biotechnology, Piscataway, NJ) was used to separate a low-density (LD) fraction (<1.077 g/mL). In some experiments, LD cells were further separated into a nonadherent LD T-lymphocyte–depleted (NALDT$^-$) fraction by adherence to plastic and removal of E-rosette–positive cells.[35] Unseparated blood cells were obtained from umbilical cord and placental tissues scheduled for discard after delivery of the infant and after prior need for samples for clinical study had been satisfied. The Human Investigation Committee of this institution has approved the collection of blood in this circumstance. Umbilical cord blood was collected as described previously.[31] Thawed umbilical cord blood was obtained from samples that had been cryopreserved as described.[31] LD umbilical cord blood fractions were obtained as described for bone marrow.

Cytokines. Purified rhu interleukin-3 (IL-3) and purified rhuGM-CSF each had specific activities in excess of 4×10^7 CFU/mg protein as assessed by half-maximal tritiated thymidine incorporation into normal human bone marrow cell proliferation assays.[30] The expression and purification of rhuMGF was as described previously for rmuMGF.[18,36] Experiments were begun with rmuMGF and were continued with rhuMGF as it became available to us. There was no difference in the quality or quantity of the primary or secondary colonies or clusters that formed in the presence of either factor. The previously mentioned cytokines were obtained from Immunex Corporation (Seattle, WA). Epo (Toyobo New York, Inc, New York, NY) was purified from human urine.

Culture system. Colony formation by CFU-GEMM and BFU-E was performed as previously described.[30,31] Bone marrow cells (LD or NALDT$^-$) or umbilical cord blood cells (LD or unseparated) were plated at a concentration of either 2.5×10^4 or 5.0×10^4 cells/mL in standard 35-mm tissue culture dishes (Corning Glass Works, Corning, NY). Cultures contained a 1-mL mixture of Iscove's modified Dulbecco's Medium (IMDM; Whittaker Bioproducts, Inc, Walkersville, MD), 1% methylcellulose (Sigma Chemical Co, St Louis, MO), 30% non–heat-inactivated fetal bovine serum (FBS; Hyclone Laboratories, Inc, Logan, UT), 5×10^{-5} mol/L 2-mercaptoethanol (Fisher Scientific Co, Fair Lawn, NJ), 2 mmol/L glutamine (GIBCO Life Technologies, Inc, Grand Island, NY), and 1 U Epo (Toyobo). In addition, cultures contained either 10% vol/vol IMDM, 100 U rhuIL-3, 50 ng rhuMGF, or 50 ng rmuMGF. Cultures were incubated at 37°C in a fully humidified atmosphere flushed with 5% CO_2 in 5% O_2 for 14 to 18 days.[37] CFU-GEMM and BFU-E colony types were assessed on whole plates using an inverted microscope as described previously.[30,35]

Colony transfer experiments. On day 14 to 18 of primary culture, single, well-isolated CFU-GEMM or BFU-E colonies were removed from primary cultures under sterile conditions using a micropipettor (Gilson P-20; Rainin Instrument Co, Emeryville, CA). Each colony, in a volume of primary culture not greater than 20 μL, was transferred to an individual tube containing 0.64 mL of culture medium and the colony was dispersed into a single-cell suspension by gentle pipetting. To this, 0.56 mL of methylcellulose culture medium was added and the culture was mixed by vortexing. (The final concentration of the components in the 1.2-mL methylcellulose culture was as described for primary cultures.) The entire content of the tube was then plated into a single 35-mm dish containing 1 U/mL Epo, 100 U/mL rhuGM-CSF, and either 50 ng/mL rhuMGF or 50 ng/mL rmuMGF using a 1-cm^3 syringe. Secondary cultures were examined with an inverted microscope immediately after plating for the presence of any cellular aggregates that remained. Few secondary cultures containing such aggregates were noted, and these were discarded. All secondary cultures were incubated for 14 days as described previously and scored for colonies (>40 cells) and clusters (5 to 40 cells) using an inverted microscope.

Statistics. Results are expressed as the mean ± 1 SEM. Levels of significance between samples were determined using the Student's *t* distribution.

RESULTS

To investigate the replating potential of human umbilical cord blood CFU-GEMM, a total of 861 cord blood colonies were cultured in the presence of Epo alone or Epo in combination with rhuIL-3 or MGF (rhu or rmu) (Table 1). Colonies were removed by hand and replated individually into secondary cultures that contained a combination of Epo, rhuGM-CSF, and either rhuMGF or rmuMGF. The replating efficiency is shown as the percentage of primary colonies giving rise to at least one secondary colony or cluster. Secondary cultures contained both CFU-GM colonies (40 to >1,000 cells) and CFU-GM clusters (5 to 40 cells) with 46% of growth in secondary cultures being of colony size. Erythroid growth on secondary plates was mainly large BFU-E clusters (<40 cells). Mixed growth was mainly CFU-GEMM colonies, but none of these secondary colonies were equal in size to the primary CFU-GEMM colonies from which they were derived. No correlation was observed between the size of replated primary colonies and replating efficiency. When cord blood CFU-GEMM colonies that had been initiated in Epo plus MGF were transferred to secondary cultures, an average of 46.3% ± 5.8% of these transfers gave rise to secondary hematopoietic colonies or clusters of multipotential (CFU-GEMM) and more differentiated (BFU-E and CFU-GM) lineages. The replating efficiency of CFU-GEMM in response to Epo plus MGF was greater than that seen with CFU-GEMM colonies growing in primary cultures in the presence of Epo alone ($P < .005$) or Epo plus rhuIL-3 ($P < .025$). More importantly, the mean number of colonies plus clusters present in all secondary cultures was sevenfold to ninefold greater when the replated CFU-GEMM colonies had been originally stimulated by Epo plus MGF compared to those grown with Epo ± rhuIL-3 ($P < .01/.005$). CFU-GEMM colonies initiated with Epo plus MGF in primary culture also gave rise to greater numbers of secondary multipotential colonies plus clusters than with Epo ± rhuIL-3 ($P < .01/.05$).

Table 2 summarizes experiments with adult human bone marrow, in which a total of 518 randomly chosen colonies containing erythroid cells (BFU-E and CFU-GEMM not being distinguished) were replated into secondary cultures.

Table 1. Replating Efficiency of Human Umbilical Cord Blood Multipotential (CFU-GEMM) Hematopoietic Progenitor Cells

Primary Colonies Grown on:	Secondary Colonies and Clusters*						
	No. Experiments	No. Primary CFU-GEMM Colonies Replated	% Replates With at Least 1 Colony or Cluster	CFU-GM per Replated Colony	BFU-E per Replated Colony	CFU-GEMM per Replated Colony	Total No. of Colonies and Clusters per Replated Colony
huEpo (1 U/mL)	10	240	27.0 ± 6.3	1.6 ± 0.5	0.3 ± 0.1	1.5 ± 0.9	3.4 ± 1.2
huEpo + rhuIL-3 (100 U/mL)	13	288	29.0 ± 5.8	3.3 ± 1.1	0.4 ± 0.3	0.5 ± 0.2	4.2 ± 1.3
huEpo + rMGF (50 ng/mL)	14	333	46.3 ± 5.8†‡	10.2 ± 3.1	1.2 ± 0.9	18.0 ± 7.1§‖	29.8 ± 7.0¶#

These results are based on six experiments with fresh unseparated cord blood cells, two experiments with fresh LD cord blood cells, and two experiments with 1- to 3-year-old thawed unseparated cord blood cells where the replating of CFU-GEMM that had been grown on Epo, Epo + IL-3, and Epo + MGF was compared. In addition, two experiments with 1- to 3-year-old thawed unseparated cord blood compared the replating of CFU-GEMM that had been cultured in Epo + IL-3 and Epo + MGF, and one experiment with 5-year-old thawed LD cord blood cells evaluated the replating of CFU-GEMM that were grown on Epo + MGF. The data obtained in replating experiments in which one or two culture conditions were compared was representative of those 10 experiments in which all three culture conditions were compared.

*Secondary colonies were grown in the presence of huEpo (1 U/mL), rhu or rmuMGF (50 ng/mL), and rhuGM-CSF (100 U/mL).

†Compared with mean for huEpo + rhuIL-3, $P < .025$.

‡Compared with mean for huEpo, $P < .005$.

§Compared with mean for huEpo + rhuIL-3, $P < .005$.

‖Compared with mean for huEpo, $P < .010$.

¶Compared with mean for huEpo + rhuIL-3, $P < .025$.

#Compared with mean for huEpo, $P < .050$.

Secondary hematopoietic colonies/clusters formed in 61.8% ± 5.7% of cultures derived from erythroid colonies that were initiated with Epo + MGF, a replating efficiency that was greater than or equal to that observed with cells cultured with Epo alone ($P < .025$) or Epo + rhuIL-3. The secondary hematopoietic colonies/clusters that resulted were mainly of the CFU-GM lineage, although occasional BFU-E developed. When randomly chosen erythroid-containing colonies were transferred to secondary cultures, rare multipotential colonies arose, and these were observed only in cultures of cells replated from colonies grown with Epo plus MGF. The mean number of secondary colonies plus clusters derived from colonies that were initiated in Epo plus MGF was fourfold to sixfold greater than those initiated in Epo alone ($P < .005$) or Epo + IL-3 ($P < .01$). Secondary colonies and clusters derived from replated erythroid-containing colonies were equal in size to those seen when umbilical cord blood CFU-GEMM colonies were transferred.

In selected experiments, adult bone marrow CFU-GEMM were identified and were evaluated for replating efficiency (Table 3). When colonies grown in Epo + MGF were transferred to secondary cultures, average replating efficiencies varied from 52.3% to 83%. The total number of colonies and clusters per replated marrow CFU-GEMM colony ranged from 4.5 to 28.9; for two of the experiments this compared with that noted in Table 1 for replated cord blood CFU-GEMM (29.8 ± 7.0). However, the secondary hematopoietic colonies and clusters that arose from replated bone marrow CFU-GEMM consisted mainly of the GM type. Rare secondary multipotential colonies and only few secondary erythroid clusters were observed in cultures of replated bone marrow CFU-GEMM colonies. This finding was in contrast to the greater number of BFU-E and CFU-GEMM colonies formed from replated umbilical cord blood CFU-GEMM (Table 1).

To compare the replating capacity of the CFU-GEMM with that of the more terminally differentiated BFU-E, we

Table 2. Replating Efficiency of Adult Human Bone Marrow Erythroid-Containing (CFU-GEMM plus BFU-E) Colonies

Primary Colonies Grown on:	Secondary Colonies and Clusters*						
	No. Experiments	No. Primary Colonies Replated	% Replates With at Least 1 Colony or Cluster	CFU-GM per Replated Colony	BFU-E per Replated Colony	CFU-GEMM per Replated Colony	Total No. of Colonies and Clusters per Replated Colony
huEpo (1 U/mL)	5	192	35.0 ± 5.6	1.8 ± 0.8	0.1 ± 0.1	0	1.9 ± 0.8
huEpo + rhuIL-3 (100 U/mL)	4	132	48.0 ± 8.2	2.5 ± 0.4	0.2 ± 0.1	0	2.7 ± 0.4
huEpo + rMGF (50 ng/mL)	5	194	61.8 ± 5.7†‡	9.8 ± 1.7	1.0 ± 0.5	0.1 ± 0.1	10.8 ± 1.5§‖

These results are based on experiments with fresh LD (N = 2) and NALDT⁻ (N = 3) bone marrow cells.

*Secondary colonies were grown in the presence of huEpo (1 U/mL), rhu or rmuMGF (50 ng/mL), and rhuGM-CSF (100 U/mL).

†Compared with mean for huEpo + rhuIL-3, not significant ($P > .05$).

‡Compared with mean for huEpo, $P < .025$.

§Compared with mean for huEpo + rhuIL-3, $P < .010$.

‖Compared with mean for huEpo, $P < .005$.

Table 3. Replating Efficiency of Adult Human Bone Marrow Multipotential (CFU-GEMM) Progenitor Cells

Primary Colonies Grown on huEpo (1 U/mL) + rMGF (50 ng/mL) Individual Experiments With:	Secondary Colonies and Clusters*					
	No. Primary Colonies Replated	% Replates With at Least 1 Colony or Cluster	CFU-GM per Replated Colony	BFU-E per Replated Colony	CFU-GEMM per Replated Colony	Total No. of Colonies and Clusters per Replated Colony
LD cells	149	52.3	4.4	0	0.1	4.5
NALDT$^-$ cells	22	82.0	28.3	0.6	0	28.9
NALDT$^-$ cells	18	83.0	18.4	0.7	0	19.1

*Secondary colonies were grown in the presence of huEpo (1 U/mL), rhu or rmuMGF (50 ng/mL), and rhuGM-CSF (100 U/mL).

replated individual umbilical cord blood and adult bone marrow BFU-E colonies (Table 4). Secondary hematopoietic colonies/clusters formed on average in 46% ± 4% of cultures derived from bone marrow BFU-E colonies that were initiated in Epo plus MGF, but the mean number of colonies plus clusters per replated BFU-E was low. Secondary colonies and clusters were entirely CFU-GM. No secondary multipotential or erythroid colonies or clusters arose when bone marrow BFU-E were replated. Numbers of secondary colonies and clusters observed when umbilical cord blood BFU-E were replated were not significantly different ($P > .05$) from bone marrow BFU-E. Secondary growth was also mainly CFU-GM, although rare erythroid clusters developed in cultures from cord blood colonies that had been stimulated by Epo alone.

DISCUSSION

In previous colony replating studies, human and murine myeloid progenitor cell "self-renewal" was evaluated in response to a variety of conditioned media.[12-14] Erythroid colony formation was stimulated by the addition of either Epo or human plasma.[12-14] The results of these studies suggested that CFU-GEMM possesses only a limited capacity for "self-renewal" based on a replating efficiency of 10% to 26%, while BFU-E were shown to have no "self-renewal" capability. Few secondary CFU-GEMM were detected in early studies of colony replatings. In contrast, our studies have shown that in response to rhu or rmuMGF, human CFU-GEMM have a greater replating (self-renewal) potential than previously described. In addition, when umbilical cord blood CFU-GEMM were replated in the current study, many CFU-GEMM were observed in our secondary cultures that contained MGF. Human BFU-E appear to be capable of limited replating, although to a lesser extent than CFU-GEMM. The BFU-E chosen for replating into secondary cultures were distinguished according to classical colony morphology and appeared to contain only hemoglobinized cells. While the limited replating efficiency of BFU-E was expected, it was of interest that the secondary growth was mainly of the GM type. The possibility exists that either the BFU-E transferred into secondary cultures were CFU-GEMM with more limited replating capacity, or that a subpopulation of BFU-E contains a small but undetected population of cells capable of nonerythroid differentiation.

We have noted in other studies that when cultures of umbilical cord blood are stimulated by optimal concentrations of rhuGM-CSF plus rhu or rmuMGF, approximately 8 to 10 times more CFU-GM are detectable than when cord blood cells are stimulated by either rhuGM-CSF or human urinary bladder carcinoma cell line-conditioned medium (5637 CM).[38] Also, when cord blood cells are grown in the presence of rhuEpo, rhuIL-3, and either rhu or rmuMGF, approximately 13 to 14 times more CFU-GEMM are detected as compared with when cord blood cells are cultured in rhuEpo and rhuIL-3. MGF also increases the detection of CFU-GM, CFU-GEMM, and BFU-E in adult bone marrow.[30] These studies suggest that the capacity of CFU-GEMM and BFU-E for replating in assays performed by others was underestimated when secondary cultures contained only conditioned medium and that the presence of MGF in our secondary cultures probably enhanced the detection of numbers of secondary colonies/clusters per replated colony.

We compared the replating capabilities of human CFU-GEMM and BFU-E present in umbilical cord and adult

Table 4. Comparative Replating Efficiency of Adult Human Bone Marrow (BM) and Umbilical Cord Blood (CB) Erythroid (BFU-E) Progenitor Cells

Primary Colonies Grown on:	Secondary Colonies and Clusters*											
	No. Primary Colonies Replated		% Replates With at Least 1 Colony or Cluster		CFU-GM per Replate		BFU-E per Replate		CFU-GEMM per Replate		Total No. of Colonies and Clusters per Replated Colony	
	BM	CB	BM	CB	BM	CB	BM	CB	BM	CB	BM	CB
huEpo (1 U/mL)	40	42	24 ± 6	27 ± 10	0.8 ± 0.1	1.4 ± 0.8	0	0.3 ± 0.2	0	0	0.8 ± 0.1	1.9 ± 0.9
huEpo (1 U/mL) + huMGF (50 ng/mL)	47	50	46 ± 4	20 ± 10	2.2 ± 0.6	2.3 ± 1.8	0	0	0	0	2.2 ± 0.6	2.3 ± 1.8

Bone marrow results are based on two experiments with NALDT$^-$ bone marrow. Cord blood results are based on three experiments with unseparated cord blood and one experiment with low-density cord blood.

*Secondary colonies were grown in the presence of rhuEpo (1 U/mL), rhuMGF (50 ng/mL), and rhuGM-CSF (100 U/mL).

bone marrow. The bone marrow is the primary site of production of hematopoietic stem and progenitor cells in the human adult.[39] The development of the human hematopoietic system involves a series of organized changes in early embryonic and fetal development. At 3 to 4 weeks postconception, the yolk sac contains the first generation of proliferating hematopoietic stem cells. At 5 to 6 weeks, the yolk sac is replaced by the fetal liver as the main hematopoietic site, and later hematopoiesis takes place in the fetal bone marrow.[39,40] During development, the fetal blood is greatly enriched in stem/progenitor cells and multipotential progenitor cells in particular, and their frequency equals or exceeds that of bone marrow.[31,33] We found no significant differences in the replating efficiencies, size, or number of secondary colonies plus clusters per replate between cord blood and bone marrow BFU-E. It is possible that other cytokines may enhance the replating capability of BFU-E. Cord blood and bone marrow CFU-GEMM colonies were equal in size, had essentially similar replating efficiencies, and the numbers and size of secondary colonies and clusters on replates did not differ much between bone marrow and cord blood CFU-GEMM. Interestingly, the secondary hematopoietic colonies and clusters that were derived from replated bone marrow CFU-GEMM were mainly of the more differentiated GM lineage. Rare CFU-GEMM were observed in cultures of replated bone marrow colonies. However, when umbilical cord blood CFU-GEMM replated they gave rise to large numbers of secondary CFU-GEMM. This difference may reflect intrinsic differences between cord blood and adult marrow CFU-GEMM or different accessory cell populations in the two tissue sources.

In summary, these colony replating studies show that cells in colonies derived from CFU-GEMM can be replated in secondary culture and that CFU-GM, BFU-E, and CFU-GEMM result. A strict definition for self-renewal events would be cellular division that resulted in daughter cells with identical capacity for proliferation and differentiation compared with the parent cell, although cells with varying degrees of stemness most likely exist. The data we report show maintenance of differentiation along multiple lineages for replated cells, which satisfies one of the two criteria for self-renewal. We found that CFU-GEMM colonies formed in secondary plates were smaller than the CFU-GEMM colonies in the primary plates from which they derived. However, it is difficult to assess the impact of manipulation of cells from primary colonies before replating and what effect this may have on the ultimate size of colonies that may develop in secondary cultures. Also, colony size is not necessarily an indication of the self-renewal capacity of the cells within the colony. Therefore, we at present favor the possibility that CFU-GEMM have a capacity for at least limited self-renewal. However, we cannot definitively rule out the possibility that the CFU-GEMM colonies found in secondary plates were derived from a more mature CFU-GEMM than those CFU-GEMM which gave rise to colonies in primary plates. Either explanation supports the concept that MGF is acting on earlier populations of progenitors. At present, it is difficult to discern whether the use of MGF merely allows detection of CFU-GEMM with a high replating efficiency, or if MGF additionally enhances the replating efficiency of these cells.

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