Detection of Human Bipotential Erythroid-Megakaryocytic Progenitors in Serum-Free Collagen Gels

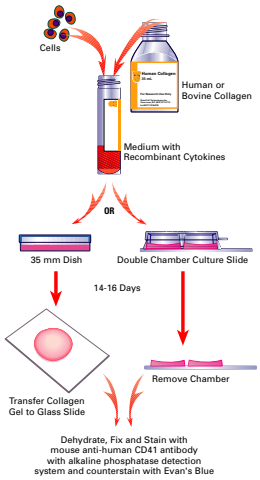
Miller CL, Faulkes S, Nielsen D, and Eaves AC
[1]StemCell Technologies Inc, Vancouver, BC, Canada; [2]Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

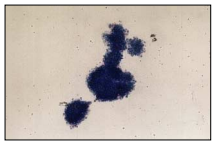
Introduction

Hematopoietic bipotential progenitors capable of differentiating into megarkaryocytes and erythroid cells (BFU-E/Mk) have been previously described[1,2] . Improved culture conditions and detection systems to distinguish bipotent and lineage restricted progenitors will facilitate future cellular and molecular biology studies on similarities and differences between the different progenitor types.

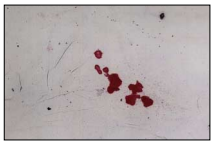
The aim of this study is to evaluate culture conditions for the optimal detection of these colony-forming cells in serum-free collagen gels. The use of culture medium lacking fetal bovine serum minimizes the potential growth inhibition of megarkaryocytic lineage cells by factors including transforming growth factor-beta (TGF-β) and platelet factor-4, commonly found in sera. *In situ* immunohistochemical staining using anti-CD41 allows definitive detection and quantitiation of BFU-E/Mk and CFU-Mk. We describe the effects of different growth factor combinations on the plating efficiency of human BFU-E/Mk.

Methods

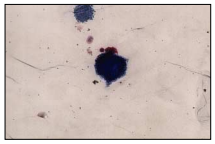




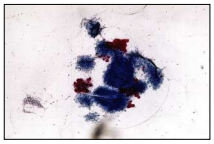
Human BFU-E
Burst forming unit erythroid:
Colony containing ≥ 200 erythroblasts



Human CFU-Mk
Colony forming unit-Megakaryocyte:
Colony containing ≥ 3 CD41$^+$ megakaryocytic cells



Human BFU-E/Mk
BFU-Erythroid-Megakaryocyte:
Colony containing erythroblasts and ≥ 3 CD41$^+$ megakaryocytic cells



Human BFU-E/Mk

Cells and Culture Conditions

CD34$^+$-enriched human bone marrow (BM) or mobilised peripheral blood (MPB) cells were isolated using StemSep™ Progenitor Enrichment Cocktail or EasySep™ CD34 Selection Cocktail. Light density (ficolled) cells and CD34$^+$-enriched cells were plated at 1-3 x10^4 and 2000-5000 cells per culture, respectively, in 1.1% collagen-based cultures supplemented with the indicated cytokines. Cultures were incubated for 12-14 days in water-jacketed incubator adjusted to 37°C, 5% CO_2 and ≥ 95% humidity. For immunohistomchemical staining, dehydrated, fixed cultures were blocked with 5% human serum in 0.05 M Tris/NaCl buffer, pH 7.6 and then sequentially treated with; 10 µg/mL mouse anti-human anti-CD41; 10 µg/mL biotin goat anti-mouse IgG antibody; 18 µg/mL avidin alkaline phosphatase conjugate; alkaline phosphatase chromogen system and counterstained with Evan's Blue.

Cytokines

Cytokines (StemCell) were used at the following final concentrations; 3 U/mL hErythropoietin (Epo), 50 ng/mL hThrombopoietin (Tpo), 50 ng/mL hStem Cell Factor (SCF), 10 ng/mL hleukemia inhibitory factor (LIF), 10 or 100 ng/mL hInterleukin-6 (IL-6), 10 or 100 ng/mL hHyper IL-6 (chimeric fusion protein of IL-6 and soluble IL-6 receptor), 10 ng/mL hIL-11, 10 ng/mL hIL-3.

StemCell Technologies
777 West Broadway, Suite 808
Vancouver · BC · Canada · V5Z 4J7
Tel: 604.877.0713 · Fax: 604.877.0704
Email: info@stemcell.com
www.stemcell.com

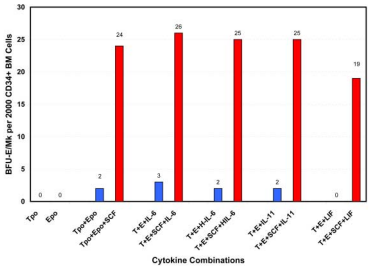
Results

Table 1. Effects of different growth factor combinations on detection of BFU-E/Mk, BFU-E and CFU-Mk

Cytokine Combination	Colonies per 2500 CD34$^+$ BM Cells		
	BFU-E/Mk	Erythroid CFCs CFU-E, BFU-E	Erythroid CFCs CFU-E, BFU-E
no cytokines	no colonies	no colonies	no colonies
3 U/mL Epo	0	48, 2	2
3 U/mL Epo, 50 ng/mL Tpo	1	4, 56	30
3 U/mL Epo, 50 ng/mL SCF	8.5	46, 84	15*
Epo, Tpo, SCF	15	66, 83	26
Epo, Tpo, SCF, 10 ng/mL IL-3	18	43, 88.5	17
Epo, Tpo, SCF, 10 ng/mL IL-6	16	43, 93	24
50 ng/mL Tpo, 10 ng/mL IL-3, 10 ng/mL IL-6	0	4	30

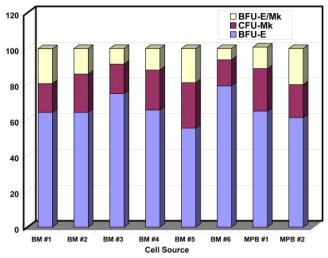
*small CFU-Mk containing ≥ 3-21 cells

Figure 1. Addition of IL-6, Hyper IL-6, LIF or IL-11 to the combinations of Epo+Tpo or Epo+Tpo+SCF did not increase the numbers of BFU-E/Mk detected.



Normal human CD34$^+$-enriched cells were cultured at 2000 cells per culture in the indicated cytokine(s). No colonies were detected in cultures containing IL-6 or IL-11 or LIF or Hyper-IL-6 only.

Figure 2. Percentages of BFU-E, CFU-Mk and BFU-E/Mk cultured in in serum-free MegaCult™-C collagen gels supplemented with Epo+Tpo+SCF.



Normal BM CD34$^+$-enriched cell populations (n=6) were cultured at 2000-4000 cells per culture and unseparated MPB (n=2) were cultured at 1x10^5 per culture for 14 days followed by immunostaining. Values represent the percentage of each CFC type of progenitors of erythoid, megakaryocyte and erythroid-megakaryocyte progenitors (BFU-E+CFU-Mk+BFU-E/Mk).

Conclusions

1. A minimal cytokine combination of Epo, Tpo and SCF is sufficient to support the proliferation and detection of human bipotential progenitors capable of differentiating into megarkaryocytes and erythroid cells (BFU-E/Mk). The frequency of BFU-E/Mk in normal bone marrow cells was found to be 8 ± 4 per 1000 CD34$^+$ cells (n=6)

2. The addition of cytokines (IL-6 or Hyper-IL-6 or IL-11) that signal through gp130, and known to promote the growth and differentiation of megakaryocytic lineage cells, did not have a significant effect on the number of BFU-E/Mk detected when added as single factors or in combination with Epo+Tpo+SCF. The effect of different growth factor combinations on the detection and plating efficiency of BFU-E/Mk requires further investigation.

3. No differences were detected in the number and morphology of BFU-E/Mk grown in bovine collagen gels compared to the use of human collagen gels (data not shown).

References

1. Bruno E, Hoffman R. 1998. Human megakaryocyte progenitor cells. Semin Hematol. 35(3):183-91.

2. Alessandro Maria Vannucchi, Francesco Paoletti, Silvia Linari, Cristina Cellai, Roberto Caporale, Pierluigi Rossi Ferrini, Massimo Sanchez, Giovanni Migliaccio, and Anna Rita Migliaccio. 2000. Identification and characterization of a bipotent (erythroid and megakaryocytic) cell precursor from the spleen of phenylhydrazine-treated mice Blood, 95: 2559-2568.