

CD34⁺

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Analysis of Stromal Cells Developed from Cord Blood CD34⁺ Cells

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= Abstract =

Background: Cytokine-mediated *ex vivo* expansion has been proposed as a means of increasing the number of cord blood (CB) hematopoietic stem cells for transplantation. As well as stem cell number, stromal cells are necessary for functional maturation of hematopoiesis. The purpose of this study was to analyze the development of stromal cells during *ex vivo* expansion of CB CD34⁺ cells. **Methods:** CD34⁺ cells were purified from CB by magnetic bead selection. The levels of interleukin-3, interleukin-1, interleukin-6, granulocyte macrophage-colony stimulating factor and tumor necrosis factor- were measured in culture supernatants on 0, 1, 2, and 3 weeks, using ELISA techniques. CB CD34⁺ cells were expanded in Iscoves modified Dulbeccos medium in the presence of several cytokines. The expression of E-selectin, vascular cell adhesion molecule-1, intercellular adhesion molecule-1, platelet/endothelial cell adhesion molecule-1, von Willebrand factor, vimentin, and CD14 in newly developed stromal cells was examined by immunocytochemical method. Relevant extracellular matrix (ECM) proteins and proper cytokines were also assayed for the most suitable condition for expansion of stromal cells. **Results:** Several cytokines were found to have been produced by CB CD34⁺ cells as well as bone marrow-derived CD34⁺ cells. During *ex vivo* expansion of CB CD34⁺ cells, stromal cells appeared in the culture by day 4 and expanded over the following 7-10 days before being confluent by day 21. These cells expressed surface markers characteristic of cells of endothelial lineage. Furthermore, these stromal cells also expanded effectively when treated with thrombopoietin+flt-3 ligand+stem cell factor+leukemia inhibitory factor or 0.1% poly-L-lysine-coated wells. **Conclusion:** Stromal cells were developed during *ex vivo* expansion of CB CD34⁺ cells and that this development could be enhanced further by treating the stromal cells with cytokines or ECM.

Key Words: Cord blood, *Ex vivo* expansion, Stromal cells, Cytokines, Extracellular matrix

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(HMP-

가
 가
 (extracellular matrices)
 가
 가
 blocking
 가
 stem cell factor (SCF),
 interleukin-3 (IL-3), interleukin-6 (IL-6), interleukin-1
 (IL-1), interleukin-7 (IL-7), granulocyte macrophage-
 colony stimulating factor (GM-CSF) tumor necrosis
 factor- (TNF-)
 fibrinogen, fibrinectin,
 hyaluronic acid, laminin
 contact/soluble signal
 apoptosis
 가
 가
 가

1.
 (14)
 (6)
 2.
 (1) CD34⁺
 Iscoves modified Dulbeccos
 medium (IMDM, Gibco, Grand Island, NY, USA)
 Ficoll-Hypaque (d=1.077, Pharmacia Bio-
 tech, Uppsala, Sweden) , x400 g 30
 IMDM 1
 pipetting
 1
 0.1% bovine serum albumin
 (BSA; Stem Cell Technologies, Vancouver, BC, Canada)
 phosphate-buffered saline (PBS, pH 7.4) 1.0
 × 10⁶ cells/300 μL 100 μL anti-
 CD34 monoclonal antibody (QBEND 10; Miltenyi
 Biotec; Bergisch Gladbach, Germany)가 colloidal
 superparamagnetic beads (Miltenyi Biotec, Glodbach,
 Germany) 가 20
 PBS 가 MiniMACS
 column (Miltenyi Biotec, Bergisch Gladbach, Germany)
 CD34⁺
 column
 CD34 , fluorescein isothiocyanate
 (FITC; HPCA-2; Becton Dickinson, Mountain View, CA,
 USA) FACSCalibur (Becton Dickinson,
 Mountain View, CA, USA)
 (2)
 (1) CD34⁺ IMDM, 12.5%
 fetal bovine serum, 12.5% horse serum, 10⁻⁴ mol/L
 2-mercaptoethanol, 10⁻⁶ mol/L hydrocortisone, 100 U/mL

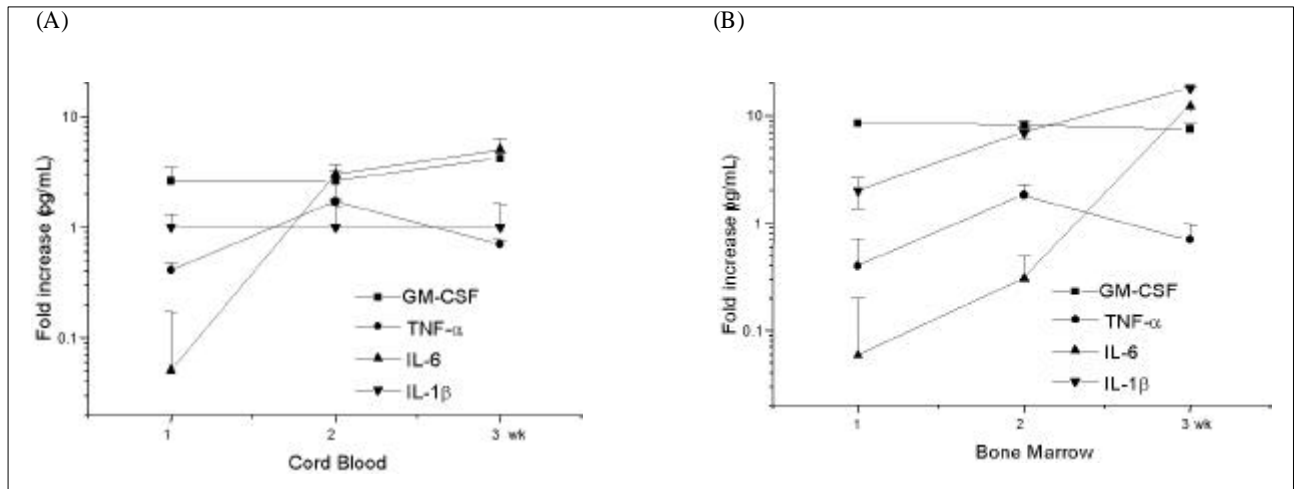


Fig. 1. The changes of cytokine levels in long-term culture media from CD34⁺ cells from cord blood (CB) and bone marrow (BM) with time. The levels of of interleukin-3, interleukin-1 (IL-1), interleukin-6 (IL-6), granulocyte macrophage-colony stimulating factor (GM-CSF) and tumor necrosis factor- were measured in culture supernatants on 0, 1, 2, and 3 weeks, using ELISA techniques. GM-CSF and IL-6 were increased with time from CB CD34⁺ cells (A). IL-6 and IL-1 were also increased with time from BM CD34⁺ cells (B).

penicillin 100 U/mL streptomycin T25
 flask (25 100 COL1; Corning, New York, NY, USA)
 10^7 /mL 가
 37°C, 5% CO₂ 가 3
 1 0, 1, 2 3
 -80°C 가 IL-3,
 IL-6, GM-CSF TNF- cytokine Endogen
 (Woburn, MA, USA), IL-1 R&D (Minneapolis,
 MN, USA) commercial kit, ELISA

(3)
 10^5 cells/mL (SFEM,
 StemCell Technologies, Vancouver, BC, Canada)
 thrombopoietin (TPO, T; 50 ng/mL, Kirin), flt-3 ligand
 (FL, F; 50 ng/mL, Chemicon), interleukin-6 (IL-6, 6; 10
 ng/mL, Endogen), leukemia inhibitory factor (LIF, L; 10
 ng/mL, Endogen), granulocyte colony-stimulating factor
 (G-CSF, G; 20 ng/mL, Endogen), stem cell factor (SCF,
 S; 50 ng/mL, Endogen) 가
 가 . 1 2
 가

collagen S (5 ug/cm², Boeringer Mannheim), fibronectin
 (5 ug/cm², Boeringer Mannheim), laminin (ug/cm²,
 Boeringer Mannheim) poly-L-lysine (5 ug/cm², Sigma)
 coating .

(4)
 poly-L-lysine coating
 (Iwaki) acetone
 . 1 E-selectin (Chemicon), vascular
 cell adhesion molecule-1 (VCAM-1; Chemicon),
 intercellular adhesion molecule-1 (ICAM-1; Chemicon),
 platelet/endothelial cell adhesion molecule-1 (PECAM-1;
 Chemicon), von Willebrand factor (vWF; Chemicon),
 vimentin (Chemicon), CD 14 (Pharmingen) .
 . 2
 FITC-goat anti-mouse Igs (Chemicon)

(5)
 \pm , Fig
 + . SPSS(Statistical Pack-
 age for Social Science)
 cytokine one way ANOVA
 P value 0.05

CD34⁺

1. CD34⁺

2.66%
가

3. CD34⁺ 가

GM-CSF
가

IL-6 (P=0.014)
TNF-
IL-1
가

(Fig. 1). IL-3
0

2. CD34⁺

CD34⁺
95%

Fig. 2

3-4 가
(Fig. 2, B-C) 7-10 가
D-E). 14-21 가
(Fig. 2, F).

3. CD34⁺

anti-CD34
CD34

vWF VCAM-1, ICAM-1,
PECAM-1, E-selectin
(Fig. 3).
CD 14 vimentin

4.

1) 가
CD34⁺

가 TPO+FL+SCF+LIF
가 가
confluent area (CA)가 가
1 3
4
(Fig. 4-A).

2) 가
CD34⁺

가 TPO+FL+SCF+LIF
가
CA 가 65±5.5% 1% poly-
L-lysine 가 91±7.8% 가
fibronectin, laminin collagen (Fig.
4-B).

(endothelial cell) (fibroblast),
network (adipocyte)
(extracellular matrix)

homing¹⁰⁾

^{11,12)}

가

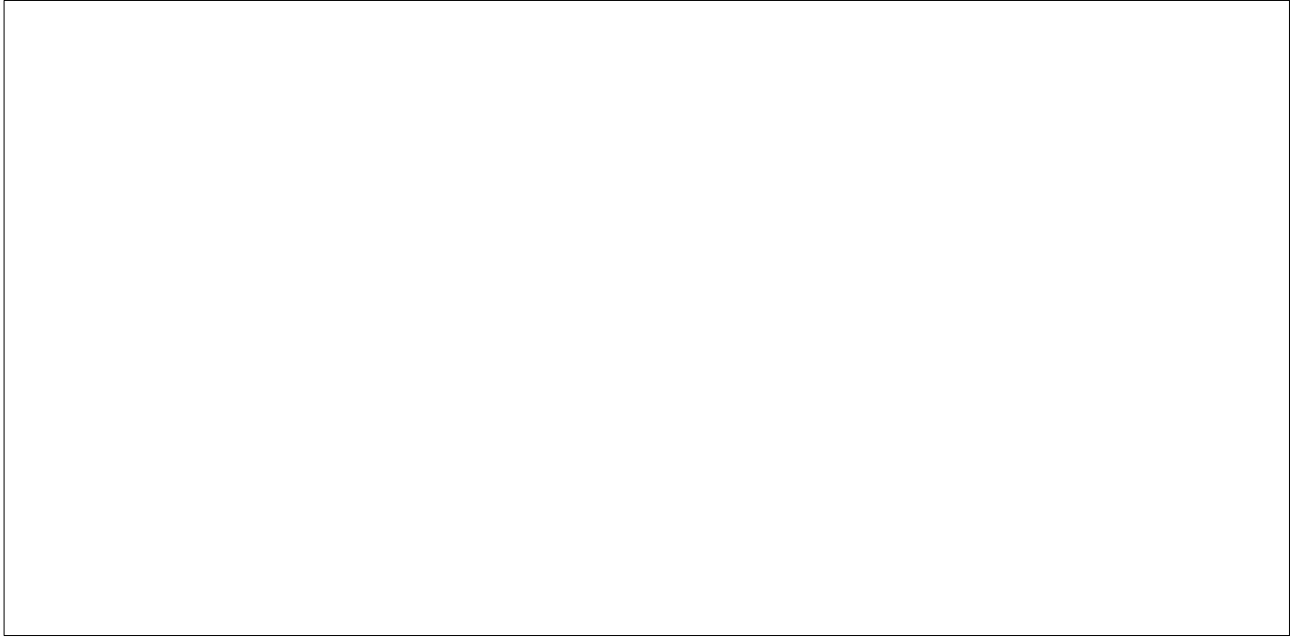


Fig. 2. The morphology of stromal cells from cord blood CD34⁺ cells during ex vivo expansion. Cord blood (CB) CD34⁺ cells were expanded in Iscoves modified Dulbeccos medium in the presence of several cytokines. During ex vivo expansion of CB CD34⁺ cells, stromal cells appeared in the culture by day 4 (A), expanded over the following 7-10 days (B-C) before being confluent by day 21 (D-E) and then adhered to hematopoietic cells (F).

가 . CD34⁺ 가 ,
 가 가
 5
 CD34⁺
 가
 CD34⁺
 13)
 CD34⁺ 가
 가 가
 2
 G-CSF, GM-CSF, IL-1 , IL-4,
 column 2 IL-5, IL-6, IL-7, IL-8, IL-11, SCF, TNF- LIF
 95% CD34⁺ 15,16) G-CSF,
 가 4 가 GM-CSF, IL-1 IL-6 , 가
 2-3 가 TNF- , transforming growth factor- (TGF-)
 interferon- (INF-) 17).
 CD34⁺ 가
 ,
 Nieda 14) . IL-3 Sensebe 18)

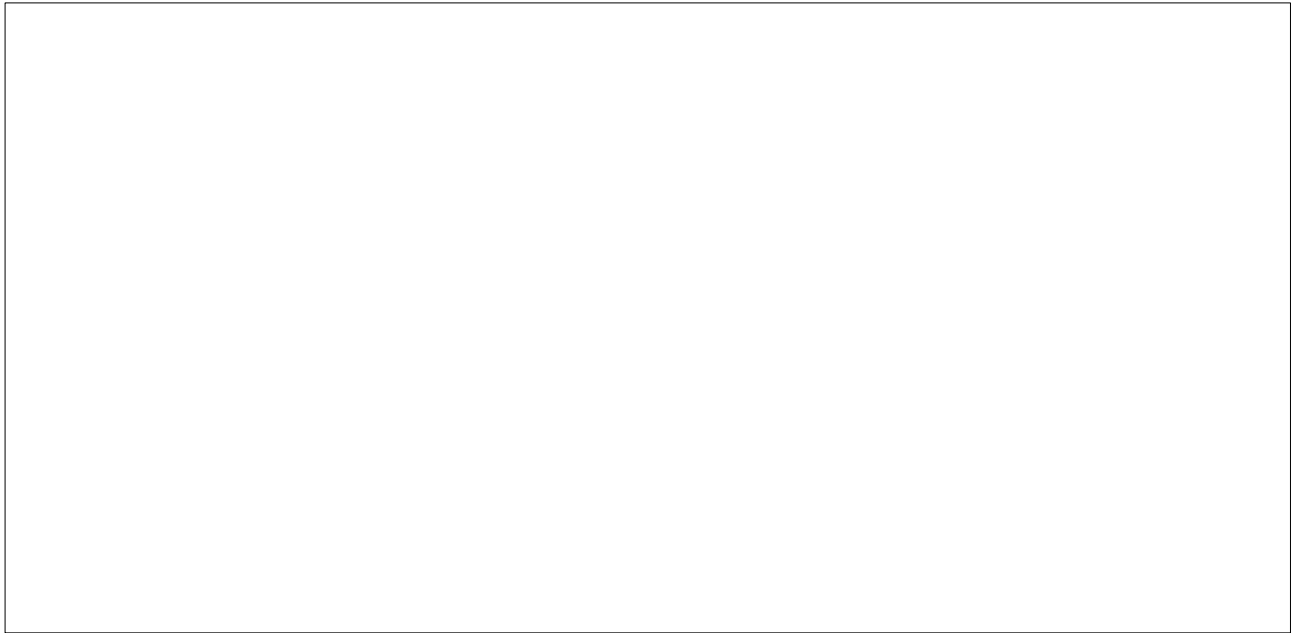


Fig. 3. The lineage markers of stromal cells from cord blood CD34+ cells. These cells were endothelial cell lineages because they were expressed positively for von Willebrand factor (B), vascular cell adhesion molecule-1 (C), intercellular adhesion molecule-1 (D), platelet/endothelial cell adhesion molecule-1 (E), E-selectin (F), but not expressed for vimentin and CD14 in immunocytochemical stain.

IL-3 homing 가
 19)
 GM-CSF IL-6
 가
 가
 가 IL-1 TNF- 가 20,21)
 가
 가
 TPO+FL+SCF+LIF
 vimentin CD 14 가 가 confluent area (CA)가 가
 vWF, PECAM-1, VCAM-1, 가
 ICAM-1 E-selectin CD34+ poly-L-lysine, collagen, laminin fibronectin 22,23)
 가
 signal TPO+FL+SCF+LIF
 가
 PECAM CD34+ 가 VLA-4 . 1% poly-L-lysine

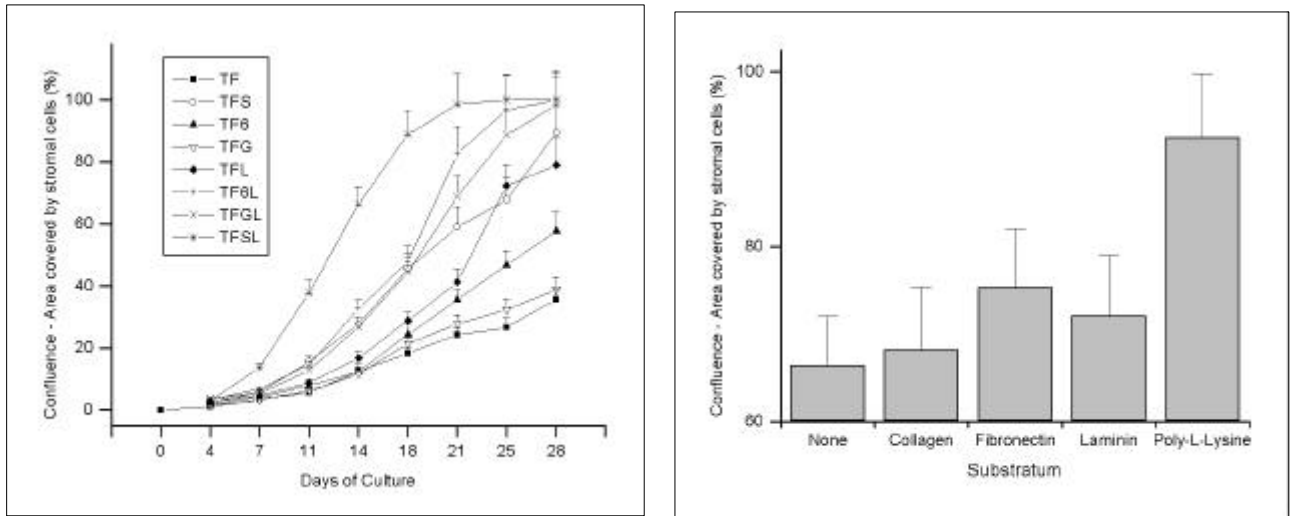


Fig. 4. The expansion of stromal cells from cord blood CD34⁺ cells during *ex vivo* expansion. Proper cytokines and extracellular matrix (ECM) proteins were assayed for the most suitable condition for expansion of stromal cells. These stromal cells were also expanded effectively with thrombopoietin+flt-3 ligand+stem cell factor+leukemia inhibitory factor (A) or 1% poly-L-lysine treatment (B). (T, thrombopoietin; F, flt-3 ligand; 6, interleukin-6; L, leukemia inhibitory factor; G, granulocyte-colony stimulating factor; S, stem cell factor)

가 가 fibronectin, laminin collagen . Gupta

24)

heparan sulfate :

가 .

CD34⁺

가

가

가

CD34⁺

collagen S, fibronectin, laminin poly-L-lysine coating CD34⁺

contact signal 가 3

, 1, 2 3 -80°C

가 IL-3, IL-6, GM-CSF, IL-1

TNF- ELISA

E-selectin, VCAM-1, ICAM-1, PECAM-1, vWF, vimentin CD 14

: CD34⁺

4 가
 7-10
 14-21
 CD34⁺ GM-CSF,
 IL-6 가
 CD34⁺
 TPO+FL+SCF+LIF 가 가
 1% poly-L-lysine
 가 :
 CD34⁺ 가
 가 가

- 1) Gluckman E: Umbilical cord blood biology and transplantation. *Curr Opin Hematol* 2; 413-416, 1995
- 2) Mayani H, Guilbert LJ, Wiecek AJ: Biology of the hematopoietic microenvironment. *Eur J Haematol* 49;225-233, 1992
- 3) Clark BR, Keating A: Biology of bone marrow stroma. *Ann NY Acad Sci* 770;70-78, 1995
- 4) Papayannopoulou T, Priestley GV, Nakamoto B: Anti-VLA-4/VCAM-1-induced mobilization requires cooperative signalling through the kit/mkit ligand pathway. *Blood* 91;2231-2239, 1998
- 5) Almici C, Carlo-Stella C, Wagner JE, Rizzoli V: Umbilical cord blood as a source of hematopoietic stem cells: from research to clinical application. *Haematologica* 80; 473-479, 1995
- 6) Gibson FM, Scopes J, Daly S, Ball EC, Smith G: Hematopoietic growth factor production by normal and aplastic anemia stroma in long-term bone marrow culture. *Br J Haematol* 91;551-561, 1995
- 7) Mayani H, Guilbert LJ, Janowska WA: Biology of the hematopoietic microenvironment. *Eur J Haematol* 49;225-233, 1992
- 8) Klein G: The extracellular matrix of the hematopoietic microenvironment. *Experientia* 51;914-26, 1995
- 9) Gupta P, Blazar BR, Gupta K, Verfaillie CM: Human CD34⁺ bone marrow cells regulate stromal production of interleukin-6 and granulocyte colony-stimulating factor and increase the colony-stimulating activity of

stroma. *Blood* 91;3724-3733, 1998

- 10) Simmons PJ, Levesque JP, Zannettino AC: Adhesion molecules in haemopoiesis. *Clin Haematol* 10;485-505, 1997
- 11) Wilson JG: Adhesive interactions in hemopoiesis. *Acta Haematol* 97;6-12, 1997
- 12) Aiuti A, Friedrich C, Sieff CA, Gutierrez-Ramos JC: Identification of distinct elements of the stromal microenvironment that control human hematopoietic stem/progenitor cell growth and differentiation. *Exp Hematol* 26;143-57, 1998
- 13) , , , , , , , : Thrombopoietin (TPO), Flt3 Ligand, Stem Cell Factor(SCF) Granulocyte-Colony Stimulating Factor(G-CSF) CD34⁺CD38⁻ ex vivo Expansion CD44 Apoptosis 2;170-180, 1997
- 14) Nieda M, Nicol A, Denning-Kendall P: Endothelial cell precursors are normal components of human umbilical cord blood. *Br J Haematol* 98;775-777, 1997
- 15) Koike M, Ishiyama T, Tomoyasu S, Tsuruoka N: Spontaneous cytokine overproduction by peripheral blood mononuclear cells from patients with myelodysplastic syndromes and aplastic anemia. *Leukemia Res* 19;639-644, 1995
- 16) Teramura M, Kobayashi S, Yoshinaga K, Iwabe K, Mizoguchi H: Effect of interleukin-11 on normal and pathological thrombopoiesis. *Cancer Chemother Pharmacol* 38;S99-102, 1996
- 17) Selleri C, Maciejewski JP, Sato T, Young NS: Interferon-gamma constitutively expressed in the stromal microenvironment of human marrow cultures mediates potent hematopoietic inhibition. *Blood* 87;4149-4157, 1996
- 18) Seensebe L, Mortensen TB, Fixe P, Herve P, Charbord P: Cytokines active on granulopoiesis: release and consumption by human marrow myeloid stromal cells. *Br J Haematol* 98;274-282, 1997
- 19) Leavesley DI, Oliver JM, Swart BW, Berndt MC, Haylock DN, Simmons PJ: Signals from platelet/endothelial cell adhesion molecule enhance the adhesive activity of the very late antigen-4 of human CD34⁺ hematopoietic progenitor cells. *J Immunol* 153;4673-4683, 1994