Medical Journal of the
Islamic Republic of Iran

Volume 18
Number 3
Fall 1383
November 2004

Basic Science in Medicine

EXPANSION OF HUMAN CORD BLOOD PRIMITIVE PROGENITORS IN SERUM-FREE MEDIA USING HUMAN BONE MARROW MESENCHYMAL STEM CELLS

M. SOLEIMANI*, H. MOZDARANI, A.A. POURFATHOLLAH*, Y. MORTAZAVI*, K. ALIMOGHADDAM***, A. HAJIFATHALI*****, M. NIKOUGOFTAR**** AND Z. ZONOBI*******

*From the * Hematology Dept., School of Medical Sciences, Tarbiat Modares University, Tehran, the ** Medical Genetics Dept., School of Medical Sciences, Tarbiat Modares University, Tehran, the*** Bone Marrow Transplantation Center, Shariati Hospital, Tehran, the**** Iranian Blood Transfusion Organization, Tehran, and ***** Shahid Beheshti Medical Sciences University, Tehran, Iran.*

ABSTRACT

Ex vivo expansion of human umbilical cord blood cells (HUCBC) is explored by several investigators to enhance the repopulating potential of HUCBC. The proliferation and expansion of human hematopoietic stem cells (HSC) in ex vivo culture was examined with the goal of generating a suitable clinical protocol for expanding HSC for patient transplantation. Using primary human mesenchymal stem cells, we established a serum-free culture system to expand human primitive progenitors and transplantable stem cells. Non-enriched cord blood $CD34^+$ cells were cultured on a monolayer of human mesenchymal stem cells in the presence of thrombopoietin (TPO), flt3/flk2 ligand (FL), and/or stem cell factor (SCF), interleukin 6 (IL-6), interleukin 3 (IL-3) under serum-free conditions. After 1 or 2 weeks of culture, cells were examined for clonogenic progenitors and percentage of $CD34^+$ CD38- cells. In the presence of TPO, FL, and SCF, fetal MSC cells supported more than a 35- and 20-fold expansion of $CD34^+$ cells and colony-forming units in culture after 1 and 2 weeks of incubation, respectively. In addition, LTC-IC assay were expanded more than 7- and 16-fold after 1 and 2 weeks of culture, respectively. UCB-HSC can be expanded in culture to numbers theoretically adequate for safe, rapid engraftment of adult patients. Additional studies are needed

Corresponding author:
H.Mozdarani, Ph.D., Dept. of Medical Genetics, School of Medical Sciences, Tarbiat Modarres University, Tehran, P.O. Box: 14115-11, Iran.
Fax: +9821-8006544
Email: mozdaranh@modares.ac.ir

to establish the functional activity of expanded UCB-HSC. This ex vivo expansion system should prove valuable in clinical settings in which stromal cells are available from recipients or stem cell donors.

MJIRI, Vol.18, No.3, 257-263, 2004.

Keywords: Ex vivo expansion; Stem cells; Umbilical cord blood; Mesenchymal stem cells.

INTRODUCTION

Umbilical cord blood (UCB) from related and unrelated donors has emerged as a novel source of stem cells for patients requiring allogeneic transplantation. [1-4] Although UCB contains hematopoietic progenitor cells at a higher frequency and with a higher proliferative capacity than adult-derived bone marrow, the low number of total hematopoietic stem cells (HSCs) contained in one UCB graft limits the potential for rapid hematological recovery in adult patients. [5,6]

In an attempt to shorten the time interval to attain donor-derived hematopoietic recovery after UCB transplantation and allow transplantation of adult recipients, phase I clinical trials have been undertaken to expand UCB in cytokines in vitro prior to infusion. [7-9] However, these clinical trials have failed to demonstrate more rapid hematopoietic recovery in UCB recipients, suggesting that cytokine-based expansion may result in differentia-tion of early self-replicative stem cells. These observa-tions corroborate recent advances in the cellular and molecular mechanisms underlying the regulation of stem cell differentiation, indicating that microenvironment or stem cell "niche" is crucial for maintenance of self-renewal capacity of stem cells. [10-14]

Mesenchymal stem cells (MSCs) are a nonhematopoietic, well-characterized homogeneous population of adherent skeletal and connective tissue progenitor cells within the bone marrow stroma. [15-17] Importantly, MSCs provide a rich environment of signals, including cytokines, extracellular matrix proteins, adhesion molecules, and cell-cell interactions, controlling the proliferation, survival, and differentiation of early lymphohematopoietic stem cells. [18,19] Studies have shown that MSCs secrete, at baseline, interleukin-6 (IL-6), IL-7, IL-8, IL-11, IL-12, IL-14, and IL-15, macrophage-colony-stimulating factor (M-CSF), flt-3 ligand (FL), and stem cell factor (SCF), similar to the cytokines and growth factors expressed by marrow-derived stromal cells. [16] Accordingly, MSCs have been shown, similar to Dexter-type stromal cells, to support long-term culture initiating cell (LTC-IC) colonies during prolonged in vitro bone marrow culture.[19] Importantly, however, IL-1α-stimulated MSCs, but not bone marrow-derived stromal cells, secrete leukemia inhibitory factor (LIF), which has been shown to inhibit embryonic stem cell differentiation. [16, 20, 21]

For these reasons, we hypothesized that the presence of a feeder layer of human MSCs during short-term UCB cytokine-based expansion may provide a "niche-like" milieu for hematopoietic stem cells and may, thus, inhibit cytokine-driven differentiation of early $CD34^+$ hematopoietic progenitor cells. We, therefore, compared expansion of UCB mononuclear cells (MNCs) during short-term culture in a combination of early-acting cytokines alone with expansion over an MSC feeder layer with added cytokines.

MATERIAL AND METHODS

Cells

UCB was obtained according to institutional guidelines from 15 normal full-term deliveries at the Shariati, Madaran and Hedayat Hospitals.

Cell processing

Buffy coat cells were obtained by centrifugation (400 g for 7 min), and low-density mononuclear cells (MNC, d<1.077 g/mL) were isolated using Ficoll-Paque Plus (Pharmacia Biotech, Uppsala, Sweden). Cells were then resuspended in Iscove Modified Dulbecco Medium (IMDM) supplemented with 2% fetal bovine serum (FBS, Gibco). Total numbers of nucleated and viable cells were determined with a hemocytometer using Turck solution and trypan blue stain, respectively.

Establishment of human mesenchymal stem cells

Bone marrow aspirates were obtained from healthy donors after informed consent. The marrow was mixed with the same volume of PBS, and mononuclear cells were separated by density gradient centrifugation. Washed cells were resuspended in stroma medium (RPMI 1640, Dutch Modification, supplemented with 10% fetal calf serum [FCS], 2 mM L-glutamine, 100 IU/mL penicillin, 0.1 mg/mL streptomycin, and 1.0 µmol/l hydrocortisone).

5×10^6 cells were placed in 80 cm^2 flasks (Nunc;

Wiesbaden, Germany) and maintained at 37°C and 5% CO_2. Half the medium was exchanged twice a week. After two weeks, confluent adherent layers were passaged with trypsin-EDTA solution 1X (Sigma-Aldrich; Steinh-eim, Germany). Trypsin was quickly inactivated with FCS-containing medium and centrifuged at 200 g for 10 min. The pellet was resuspended in stromal medium, and the cells of one 80 cm^2 culture vessel were transferred in six 25 cm^2 flasks or six six-well plates for further expansion experiments. Second passage MSCs were seeded at 3 x 10^3 cells/cm^2, grown to confluency in Dulbecco's modified Eagle's medium with 10% fetal bovine serum (FBS) (GIBCO Life Technologies; Gaithersburg, MD), and irradiated (15 Gy, ^{137}Cs irradiation) prior to co-culture with UCB, to prevent MSC overgrowth (Figure 1).

UCB Expansion Cultures

MNCs (2 x 10^6 cells/mL) from UCB were plated in StemSpan serum free medium supplemented with 10% FBS, L-glutamine, penicillin, and streptomycin. Cells were either expanded for 12 days in cytokines, as previously described[23], or in parallel in the same cytokines over a monolayer of MSCs. Cytokines included: TPO (50 ng/mL), IL-3 (50 ng/mL), IL-6 (50 ng/mL), Flt-3 (50 ng/mL) and SCF (50 ng/mL). Every third day, half-media changes were performed to replenish cytokines, and the total volume was adjusted to maintain the cells at 2 x 10^6 cells/mL. After 12 days, cultures were harvested by trypsinization to include cobblestone-forming cells embedded in the MSC layer.

Cytokines

Recombinant human flt-3 ligand (FL), recombinant human thrombopoietin (TPO), recombinant human stem cell factor (SCF, also known as mast cell factor [MGF] or c-kit ligand [KL]), recombinant human interleukin-6 and interleukin-3 were used in this study. All these cytokines were purchased from Stemcell Technology, Vancouver, BC, Canada.

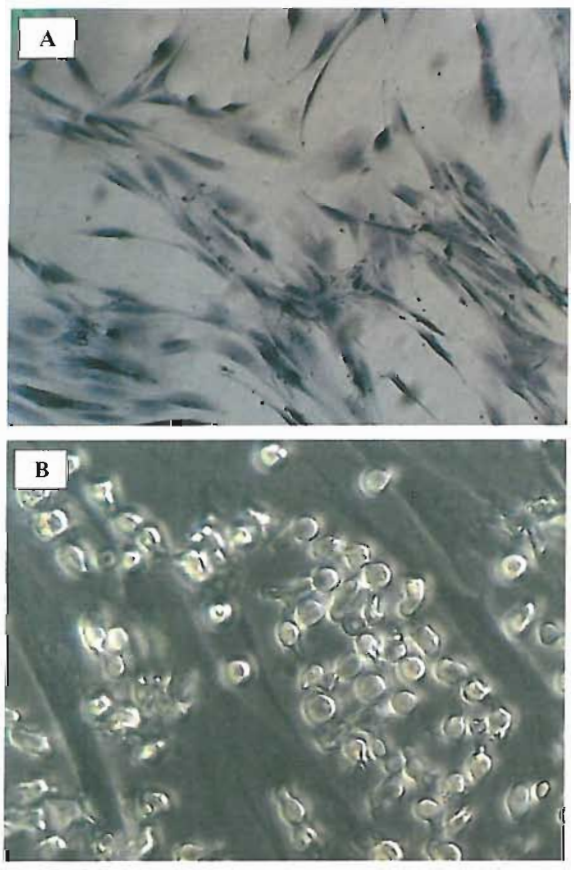


Fig. 1. (A) Phase microscopy of human mesenchymal stem cells staining by H&E. **(B)** Appearance of proliferating cells in the coculture of human cord blood $CD34^+$ cells on a monolayer of mesenchymal stem cells in the presence of TPO, SCF,IL-3, IL-6 and FL.

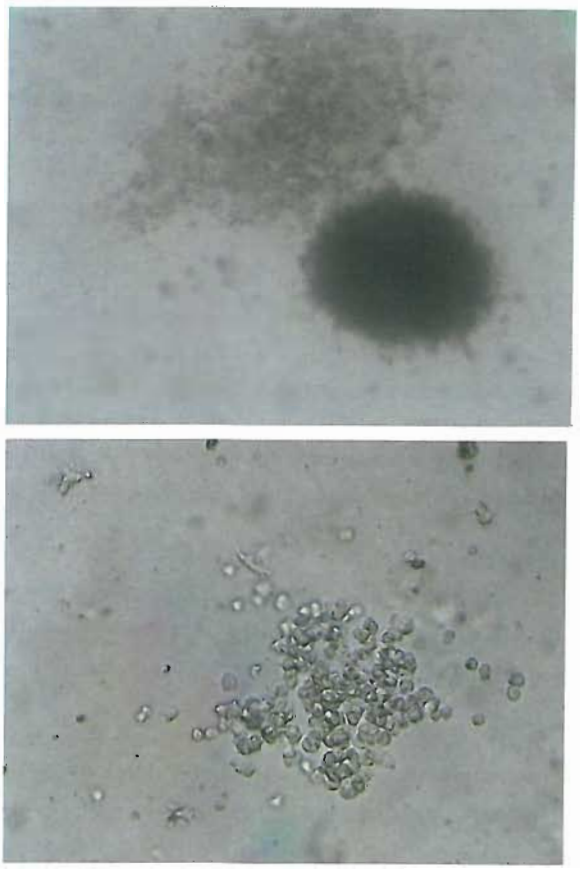


Fig. 2. Total colony-forming units in culture (CFU-C) , mixed colonies containing erythroid and myeloid cells and megakaryocytes (CFU-GEMM), CFU-GM and BFU-E consisting of 50 or more cells were scored on an inverted microscope at 14 days of culture.

Coculture of cord blood hematopoietic cells and human mesenchymal stem cell (MSCs)

MSCs cells (2,000 or 6.0 × 10^5) were plated in 25- or 75 cm^2 flasks, respectively, in 5 or 20 mL of DMEM with 10% FBS supplemented antibiotics and left for one week at 37°C in a humidified atmosphere of 5% CO_2/95% air . On the day of coculture, the MSCs cells were washed with and recultured in the serum-free Stemspan medium (Stemcell Company, Canada) and then gamma irradiated with ^{137}Cs at a dosage of 1,500 cGy irradiated feeder cell trypsinated and ten thousand cells transfer to 96-well microplate (nunc). 2×10^4 mononuclear cells were cultured on monolayer preestablished in 96-well microplate using 100μL of Stemspan medium supplemented with combinations of human TPO (50 ng/mL, Stemcell Company, Canada), human SCF (50 ng/mL), and human FL (50 ng/mL), IL-6 (50 ng/mL) and IL-3 (50 ng/mL) at 37°C in a humidified atmosphere of 5% CO_2/95% air for 2 weeks. Culture medium was replaced after 1 week of culture with fresh medium containing cytokines.

Hematopoietic Colony Assay

Methylcellulose clonal culture was performed in 35-mm suspension culture dishes. The culture medium consisted of IMDM, 1.0% 4,000-centipoise methylcellulose (Sigma), 30% FCS, 1% bovine serum albumin (BSA; Sigma Chemical Corp.), 10 ng/mL human IL-3, 10 ng/mL human SCF, 10 ng/mL G-CSF, and 2 U/mL human erythropoietin. After 14 days of incubation at 37°C in a humidified atmosphere of 5% CO_2 in air, the colonies were scored with an inverted microscope. Densely packed colonies that reached >1 mm in size were scored as high-proliferative potential colonies (HPP-CFC)[6]. The CFU-GM and BFU-E colonies are shown in Figure 2.

Functional assays

To determine their content in CFU-GM, BFU-E, and CFU-GEMM, cells were plated in complete semisolid medium under standard conditions using the following inocula: whole cord blood: 10 μL; CB-MNC: 10^4 MNC.

After 14 days of incubation at 37°C in a humified 5% CO_2 atmosphere, colonies were scored using an inverted microscope applying standard criteria for their identification. After scoring, colonies were pooled and washed, and the cells were counted using a cytometer. The total cell count was divided by the colony number to provide a median value of the cell number in each colony.

Using standard conditions, LTC-IC assays were performed by seeding an aliquot of light density cells in dishes over irradiated (1,500 cGy) human bone marrow stromal cells. LTC-IC were maintained for 5 weeks at 37°C and fed weekly by replacement of half of the growth medium (plus 10^{-6} mol/L hydrocortisone) containing half of the nonadherent cells with fresh growth medium. After 5 weeks, adherent cells were trypsinized and combined with the nonadherent fraction. These harvested cells were washed and aliquots were assayed for clonogenic precursors in standard methylcellulose culture. The value provides a relative measure of the number of LTC-IC present in the original sample input.

Flow cytometric analysis

Aliquots of cells were stained with fluorescein isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies in phosphate-buffered saline (PBS)/0.1% BSA at 4°C for 30 minutes. Analysis was performed by using an EPICS XL flow cytometer (Coulter, Tokyo, Japan). Antibodies used were as follows: FITC-conjugated CD34 antibody, and PE-conjugated CD38(DAKO). FITC- and PE-conjugated mouse IgG1 antibodies (Dako) were used as isotype-matched controls.

Statistical Analysis

For most parameters, median and range are provided. Experiments evaluating different conditions were compared using the Wilcoxon-signed rank test for paired samples. Statistical significance was assumed when the two-tailed p value was below 0.05.

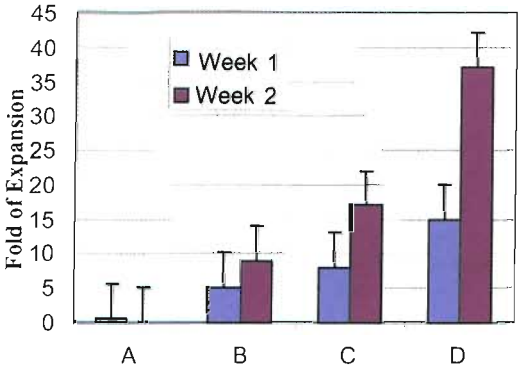
RESULTS

Synergistic effects of MSCs and early-acting cytokines on ex vivo expansion of human CB progenitors

We first examined the hematopoiesis-supporting effects of MSCs cells. $2x10^4$ MNCs cells from cord blood were plated on a MSCs cell layer under serum-free conditions with or without combinations of TPO, SCF, IL-3, IL-6 and FL. Cells not adhering and adhering weakly to MSC cells were collected by gentle pipetting after 2 weeks of culture for analysis. Without cytokines, the mean number of total nucleated cells, $CD34^+$ cells, and $CD34^+$ $CD38^-$ cells after 2 weeks of culture was 2–5 times the initial input number. In contrast, in the presence of TPO, not only total nucleated cells, $CD34^+$ cells, and $CD34^+CD38^-$ cells, but also CFU-C, CFU-Mix, and HPP-CFC were significantly expanded. The addition of SCF and/or FL to TPO further enhanced the expansion of nucleated cells and progenitors. Representative photomic-

rographs of MSCs cells and growing hematopoietic cells in culture supplemented with TPO, SCF, IL-3, IL-6 and FL are shown in Figure 2. Although there were some differences among experiments as to the degree of expansion, the maximum output of progenitors was consistently observed when stimulated with TPO, SCF, IL-3, IL-6 and FL. The results of four experiments are shown in Figure 3.

Expansion of cord blood progenitors



A: Control, B: Feeder, C: SCF, TPO, Flt-3, IL-3, IL-6, D: Feeder + cytokines

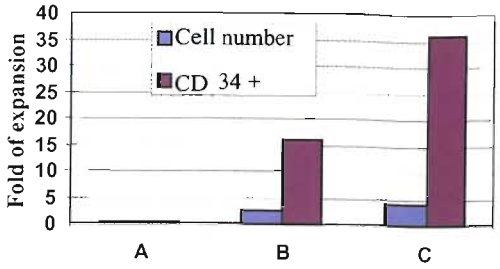
	Control	Feeder	SCF,TPO,FLt-3,IL-6,IL-3	Feeder + Cytokines
Week 1	0.5± 0.2	5 ± 2.8	8 ± 3.2	15 ± 5.1
Week 2	0	9 ± 3.33	17 ± 5.26	37 ± 9.65

Fig. 3. Expansion of human cord blood progenitors. 2×10^5 human cord blood mononuclear cells were plated on a monolayer of human MSCs in the presence of combinations of cytokines (one culture for each group). After 2 weeks of culture, growing nonadherent hematopoietic cells and cells weakly attached to MSCs were collected by gentle pipetting and analyzed by flow cytometry and colony assay. Data represent mean±SD of the fold increase compared with the initial value in four experiments performed on 13 separate cord blood donors.

Dependency of progenitor expansion on MSCs cells

We next examined whether MSCs cells are required for progenitor expansion. Mononuclear cells were cultured with TPO, SCF, IL-3, IL-6 and FL in the presence or the absence of MSC cells for 2 weeks, and growing cells were collected by gentle pipetting for analysis. The cellular proliferation was 2-fold less and the expansion of $CD34^+$ cells was 10-fold less in the absence of MSC cells than in their presence. Furthermore, $CD34^+CD38^-$ cells were only scarcely recovered without MSC cells, indicating important roles for MSC cells in progenitor expansion. The results are presented in Figure 4A. To assess whether cell–cell contact between MSC cells and progenitors are important, we examined the

Dependency of Stem/Progenitor Cells Expansion on MSCs



A: Control, B: SCF, TPO, Flt-3, IL-3, IL-6, C: MSCs feeder + Cytokines

Fig. 4A.

	A	B	C
Cell number	0.4 ± 0.2	2.7 ± 0.7	4.1 ± 1.2
CD 34+	0.4 ± 0.2	16 ± 3.4	36 ± 7.9

Effect of separation of MSCs and cord blood mononuclear cells by filter

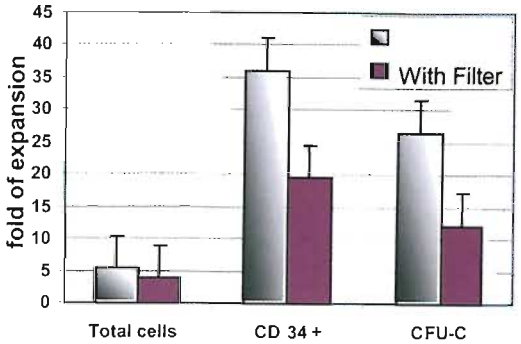


Fig. 4B.

Without Filter		With Filter
Total cells	5.3± 1.4	3.8± 1.2
CD 34+	36 ± 7.9	19.5± 5
CFU-C	22.5 ± 5	12 ± 3.8

Fig. 4. Effects of MSCs cells on Stem/Progenitor expansion. **(A)** 2×10^5 human cord blood mononuclear cells were cultured with TPO, SCF, FL, IL-3 and IL-6 in the presence or the absence of MSCs cells (one culture for each group). After 2 weeks of culture, nonadherent hematopoietic cells and cells weakly attached to MSCs cells were collected for analysis by gentle pipetting from the culture with MSCs cells, while all the cells from the culture without MSCs cells were utilized for analysis. Data represent mean ± SD of fold increase compared with the cells at the start in three experiments performed on five separate cord blood samples. **(B)** 2×10^5 human cord blood mononuclear cells were cultured on a monolayer of MSCs cells with TPO, SCF, FL, IL-3 and IL-6 for 2 weeks in the presence (noncontact) or the absence (contact) of a microporous filter (one culture for each group). After 2 weeks of culture, nonadherent hematopoietic cells and those weakly attached to MSCs cells were collected for analysis by gentle pipetting from "contact" culture, while all the cells from "noncontact" culture were utilized for analysis. A summary of three experiments performed on five separate cord blood samples is presented.

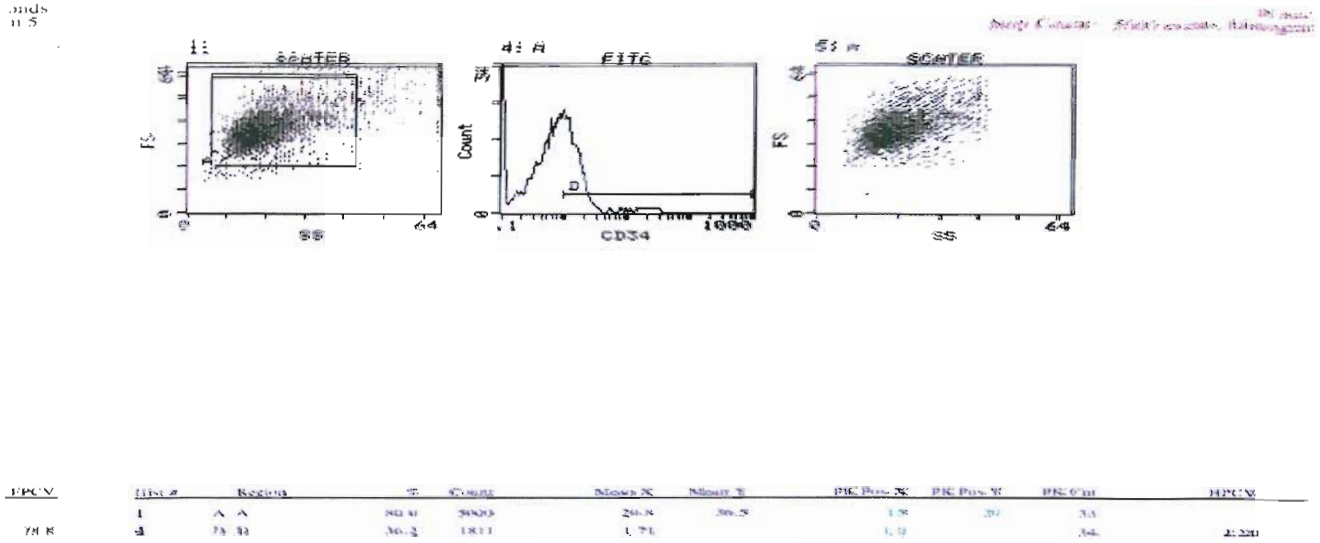


Fig. 5. Flow cytometric profile of expanded cells. Expanded cells, nonadherent hematopoietic cells, and cells attached weakly to mesenchymal stem cells were collected after 2 weeks of culture with mesenchymal stem cells, TPO, SCF, FL, IL-3 and IL-6 for flow cytometric analysis.

effects of separation of MSC cells and mononuclear cells by a 0.45-μm microporous filter. As seen in the results shown in Figure 4B, the filter separation suppressed not only total cellular proliferation, but also the expansion of $CD34^+$ cells, $CD34^+CD38^-$ cells, and CFU-C. These results suggested that diffusible soluble factor(s) generated by MSC cells were not enough for the maximum expansion of progenitors.

Flow cytometery

Representative data of flowcytometric analysis of the cells at the start of culture and after 2 weeks of expansion culture are shown in Figure 5.

DISCUSSION

Recent reports have shown that extrinsic signals, such as soluble factors and adhesion/matrix proteins provided by the stem cell microenvironment or stem cell "niche," play a crucial role in the asymmetric division of stem cells and maintenance of the stem cell pool. [10–14, 24, 25] Accordingly, several groups have reported expansion of hematopoietic progenitors in the presence of stromal elements and other types of feeder layers, with varying outcomes. [26–28]

Human MSCs not only provide a rich environment of cytokines, extracellular matrix proteins, and adhesion molecules, but also, unlike stromal cells, produce LIF, a cytokine that has been shown to maintain embryonic stem cells in a self-replicative state. [20, 21] Moreover, it has been shown that hematopoietic progenitor cells cultured on MSCs form cobblestone areas reaching deep into the MSC layer, suggestive of establishment of close contact and a niche-like environment. [16] For these reasons, we studied human MSCs as a feeder layer during standard cytokine-based in vitro UCB expansion conditions, and showed here a clear benefit of MSCs, with increased $CD34^+$, CFU, and LTC-IC counts, above unexpanded cells, as well as greater expression of regulatory proteins critical to HSC survival and maintenance of self-replicative capacity, compared with cells expanded in cytokines alone.

These studies, designed to compare in vitro readouts of UCB cultured for 12 days in the presence of cytokines with or without MSCs as a feeder layer, support the hypothesis that MSCs may preserve early $CD34^+$ progenitor potential by reducing differentiation-induced cell-cycle progress and apoptosis, and may, thus, contribute to the preservation/expansion of this early progenitor cell pool.

REFERENCES

1. Laughlin MJ, Barker J, Bambach B, et al: Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors. N Engl J Med 344: 1815–1822, 2001.
2. Rubinstein P, Carrier C, Scaradavou A, et al: Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med 339: 1565–1577, 1998.

3. Kurtzberg J, Laughlin M, Graham ML, et al: Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med 335: 157–166, 1996.
4. Gluckman E, Rocha V, Boyer-Chammard A, et al: Outcome of cord-blood transplantation from related and unrelated donors. Eurocord Transplant Group and the European Blood and Marrow Transplantation Group. N Engl J Med 337: 373–381, 1997.
5. Paulin T: Importance of bone marrow cell dose in bone marrow transplantation. Clin Transplant 6: 48–54, 1992.
6. Rocha V, Wagner Jr JE, Sobocinski KA, et al: Graft-versus-host disease in children who have received a cord-blood or bone marrow transplant from an HLA-identical sibling. Eurocord and International Bone Marrow Transplant Registry Working Committee on Alternative Donor and Stem Cell Sources. N Engl J Med 342: 1846–1854, 2000.
7. Pecora AL, Stiff P, Jennis A, et al: Prompt and durable engraftment in two older adult patients with high risk chronic myelogenous leukemia (CML) using ex vivo expanded and unmanipulated unrelated umbilical cord blood. Bone Marrow Transplant 25: 797–799, 2000.
8. Kogler G, Nurnberger W, Fischer J, et al: Simultaneous cord blood transplantation of ex vivo expanded together with non-expanded cells for high risk leukemia. Bone Marrow Transplant 24: 397– 403, 1999.
9. Koller MR, Manchel I, Maher RJ, et al: Clinical-scale human umbilical cord blood cell expansion in a novel automated perfusion culture system. Bone Marrow Transplant 21: 653–663, 1998.
10. Gupta P, Oegema Jr TR, Brazil JJ, et al: Human LTC-IC can be maintained for at least 5 weeks in vitro when interleukin-3 and a single chemokine are combined with O-sulfated heparan sulfates: requirement for optimal binding interactions of heparan sulfate with early-acting cytokines and matrix proteins. Blood 95: 147–155, 2000.
11. Kiger AA, White-Cooper H, Fuller MT: Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature 407: 750–754, 2000.
12. Tran J, Brenner TJ, DiNardo S: Somatic control over the germline stem cell lineage during Drosophila spermatogenesis. Nature 407: 754–757, 2000.
13. Xie T, Spradling AC: A niche maintaining germ line stem cells in the Drosophila ovary. Science 290: 328–330, 2000.
14. Kiger AA, Jones DL, Schulz C, et al: Stem cell self-renewal specified by JAK-STAT activation in response to a support cell cue. Science 294: 2524–2545, 2001.
15. Haynesworth SE, Baber MA, Caplan AI: Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies. Bone 13: 69–80, 1992.
16. Majumdar MK, Thiede MA, Mosca JD, et al: Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells. J Cell Physiol 176: 57–66, 1998.
17. Pittenger MF, Mackay AM, Beck SC, et al: Multilineage potential of adult human mesenchymal stem cells. Science 284: 143–147, 1999.
18. Haynesworth SE, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. J Cell Physiol 166: 585–592, 1996.
19. Majumdar MK, Thiede MA, Haynesworth SE, et al: Human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages. J Hematother Stem Cell Res 9: 841–848, 2000.
20. Gough NM, Williams RL, Hilton DJ, et al: LIF: a molecule with divergent actions on myeloid leukaemic cells and embryonic stem cells. Reprod Fertil Dev 1: 281–288, 1989.
21. Murray P, Edgar D: The regulation of embryonic stem cell differentiation by leukaemia inhibitory factor (LIF). Differentiation 86: 227–234, 2001.
22. Kadereit S, Mohammad SF, Miller RE, et al: Reduced NFAT1 protein expression in human umbilical cord blood T lymphocytes. Blood 94: 3101–3107, 1999.
23. Conneally E, Cashman J, Petzer A, et al: Expansion in vitro of transplantable human cord blood stem cells demonstrated using a quantitative assay of their lympho-myeloid repopulating activity in nonobese diabetic-SCID/SCID mice. Proc Natl Acad Sci USA 94: 9836–9841, 1997.
24. Verfaillie CM: Soluble factor(s) produced by human bone marrow stroma increase cytokine-induced proliferation and maturation of primitive hematopoietic progenitors while preventing their terminal differentiation. Blood 82: 2045–2053, 1993.
25. Clausen J, Stockschlader M, Fehse N, et al: Blood-derived macrophage layers in the presence of hydrocortisone support myeloid progenitors in long-term cultures of CD34+ cord blood and bone marrow cells. Ann Hematol 79: 59–65, 2000.
26. Verfaillie CM: Direct contact between human primitive hematopoietic progenitors and bone marrow stroma is not required for long-term in vitro hematopoiesis. Blood 79: 2821–2826, 1992.
27. Shimakura Y, Kawada H, Ando K, et al: Murine stromal cell line HESS-5 maintains reconstituting ability of ex vivo-generated hematopoietic stem cells from human bone marrow and cytokine-mobilized peripheral blood. Stem Cells 18: 183–189, 2000.
28. Kusadasi N, Koevoet JL, van Soest PL, et al: Stromal support augments extended long-term ex vivo expansion of hemopoietic progenitor cells. Leukemia 15: 1347–1358, 2001.