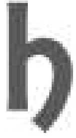

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Short-term *ex vivo* expansion sustains the homing-related properties of umbilical cord blood hematopoietic stem and progenitor cells

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A B S T R A C T

Background and Objectives. The homing of stem cells to the bone marrow microenvironment following transplantation is a specific movement eventually leading to the stem cells lodging in specialized niches of hematopoiesis. The present study was designed to develop an *ex vivo* expansion system capable of preserving the homing potential of hematopoietic stem/progenitor cells (HSPC).

Design and Methods. Umbilical cord blood (UCB) CD34$^+$ cells were expanded in QBSF-60 serum-free medium with a simple early-acting combination of cytokines and were re-selected from the expanded products at different time points. The homing-related characteristics and expansion rate of CD34$^+$ cells were simultaneously examined.

Results. It was observed that the number of HSPC increased significantly under our expansion protocol. The expression of CD49d, CD44, CD11a and CD49e on expanded CD34$^+$ cells increased or remained at the same levels as those on freshly isolated CD34$^+$ UCB cells, while the expression of CD54 on expanded CD34$^+$ cells was lower during the second week of culture than at the start. The spontaneous and SDF-1-induced adhesion of CD34$^+$ cells was increased during the first 10 days of culture, with the adhesion rates reaching peak levels (62.8±12.8% and 90.5±11.7% for spontaneous and induced adhesion, respectively) on day 10. Neither spontaneous nor SDF-1-induced migration had changed significantly by day 7.

Interpretation and Conclusions. These data demonstrate that, although *ex vivo* expansion may alter cell properties, our one-week expansion protocol can preserve most of the homing-related characteristics and activities of UCB HSPC.

Key words: CD34$^+$ cells, cord blood, *ex vivo* expansion, adhesion molecule expression, homing, transmigration efficiency, CXCR4.

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The use of umbilical cord blood (UCB) for allogeneic transplantation in adults has been hindered by the concern that a single collection may contain insufficient numbers of hematopoietic stem and progenitor cells (HSPC) to reconstitute hematopoiesis in heavier patients within an adequate time.[1-2] *Ex vivo* expansion of primitive HSPC might be a way to circumvent this problem. A good expansion system should maintain the ability of HSPC to proliferate and self-renew, and to home to the bone marrow microenvironment. Several *ex vivo* expansion protocols have been extensively evaluated and although these can sustain the proliferation and self-renewal potential of UCB primitive stem cells, little is known about the ability of expanded cells to provide long-term engraftment.[3-5] The long-term engraftment of HSPC and subsequent reconstitution of normal hematopoiesis can only occur if the HSPC home to specialized niches of the bone marrow microenvironment. Although the mechanism of *homing* of HSPC is still not completely understood, it is believed that two sequential, important processes are involved: adhesion to and migration across the endothelial layer. HSPC migrate through the endothelium into the hematopoietic compartment after adhering to the vessel wall and expressing β1 or β2 integrins such as very late activation antigen-4 (VLA-4, CD49d), VLA-5 (CD49e) and lymphocyte function associated antigen-1 (LFA-1, CD11a).[6-15] In addition, the non-integrin adhesion receptor, lymphocyte homing-associated adhesion molecule (HCAM, CD44[16] and stromal cell-derived factor (SDF-1)[17,18] also play important roles in engraftment. We have previously shown that QBSF-60 serum-free medium, with a

simple early-acting cytokine combination of Flt3 ligand (FL), stem cell factor (SCF) and thrombopoietin (TPO) $\pm$ interleukin-3 (IL-3) supports expansion of various fresh and previously frozen UCB HSPC populations involved in the different phases of engraftment. *Ex vivo*-expanded CD34$^+$ cells can retain the *in vitro* proliferation and self-renewal potential of fresh unexpanded CD34$^+$ cells.[19] These observations prompted us to investigate the homing-related characteristics of HSPC expanded using the above-described system.

Design and Methods

Preparation of cells from umblical cord blood

Human UCB samples were collected, with informed consent, from healthy full-term neonates immediately after delivery. The blood was collected by gravity into a 450 mL blood donor set containing 28 mL citrate-phosphate-dextrose-adenosine (CPD-A) anticoagulant. Nucleated cells were separated by sedimentation with 1.2% (final wt/vol) hydroxyethyl starch in 0.9% sodium chloride (Hespan, Dupont) and then the low-density fraction of mononuclear cells (MNC) was collected after standard separation on Ficoll-Paque.

Purification of CD34$^+$ cells

The CD34$^+$ cells were isolated with the miniMACS immunomagnetic separation device using a CD34 isolation kit (Miltenyi Biotch Inc., Auburn, CA, USA) according to the manufacturer's instructions. To increase their purity, cells in the CD34$^+$ fraction were applied to a second column and the purification steps repeated. MACS purification produced a 95% to 98% pure CD34$^+$ cells preparation.

Expansion culture

On day 0, enriched CD34$^+$ cells (2.0×10^4/mL) were cultured in a 25 cm^2 vented flask in 5.0-10.0 mL serum-free medium QBSF-60 (kindly provided by Quality Biological Inc., Gaithersburg, MD, USA) containing SCF 50 ng/mL (Sigma, St. Louis, MO, USA), FL 100 ng/mL (kindly provided by Immunex Co., Seattle, WA, USA), TPO 100 ng/mL (StemCell Tech Inc., Vancouver, Canada), and IL-3 20 ng/mL (StemCell Tech Inc., Vancouver, Canada). On day 4, equal volumes of fresh media were supplemented with the same doses of FL and TPO (100 ng/mL) and half doses of SCF (25 ng/mL) and divided into two flasks. Fresh media with the same cytokines as same as those on day 4 were exchanged at flexible volume to keep the cell density at about 2.0×10^5/mL after feeding on day 7 and day 10, and CD34$^+$ cells were re-selected from the expanded products on day 7, day 10 and day 14. At each time point, the expression of various cell adhesion molecules (including CD49d, CD49e, CD44, CD54 and CD11a), the adhesive and migratory features, and the expression of CXCR4 on the re-selected CD34$^+$ cells were assessed and compared with those of the initial, freshly isolated CD34$^+$ cells.

Flow cytometry assay

The expression of adhesion molecules and CXCR4 on CD34$^+$ cells was analyzed using dual-color flow cytometry. The CD34$^+$ cells (1.0×10^5) were labeled with fluorescein (FITC) or phycoerythrin (PE)-conjugated anti-CD34 (clone HPCA-2; Becton-Dickinson), PE- conjugated anti-CXCR4 (clone 12G5; R&D Systems Inc.), PE-conjugated anti-CD11a (clone HI111; Pharmingen), PE-conjugated anti-CD49e (clone IIA1, Pharmingen), PE-conjugated anti-CD54 (clone B-H17, Diaclone), FITC-conjugated anti-CD44 (clone B-A11, Diaclone), and FITC-conjugated anti-CD49d (clone BU49, Southern Biotechnology Associates). Negative controls were stained with isotype-matched FITC or PE-conjugated IgG, and compensation was adjusted using single-stained cell samples. The dual-color antibody marker analysis was performed on a FACScalibur flow cytometry configured with CellQuest software (Becton-Dickinson).

Adhesion assay

The adhesion activity of CD34$^+$ cells was assayed as previously described with minor modifications.[20] Briefly, 96-well tissue culture plates were coated with 50 µL/well PBS containing 20 µL/mL human fibronectin and incubated overnight at 4°C. The culture plates were washed three times with adhesion buffer [0.5% BSA in Iscove's modified Dulbecco's medium (IMDM)], and blocked with IMDM containing 2% BSA for 2 hours at 37° C. Freshly enriched or re-selected CD34$^+$ cells were added to the wells (1×10^4 cells/well in 100 µL adhesion buffer with or without 100 ng/mL SDF-1), allowed to adhere for 1 hour at 37°C, and then washed three times carefully with adhesion buffer to removed non-adherent cells. Subsequently, the adherent cells were stained with MTT reagent (Sigma) and the absorbency was read at 570 nm. Background cell adhesion to fibronectin was subtracted from all readings. The percentage of cells that bound specifically to the fibronectin (FN) coating was determined as follows: (A_{570} of bound cells – A_{570} of FN-coat plate)/(A_{570} of 1×10^4 cells applied– A_{570} of FN-coat plate) $\times$ 100%.

Chemotactic assays

All assays were performed in triplicate using 5-µm pore transwell filters (24-well, cluster; Costar, Corning Inc., USA). The 5 µm pore transwell filters have been

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reported to be optimal for migration of Mo7e and CD34$^+$ cells in response to chemoattractants with low background migration.[21] Before each assay, filters were rinsed in chemotaxis buffer (IMDM, 0.5% BSA) and the supernatant was aspirated. The 100 μL chemotaxis buffer containing 1×10^5 cells was added to the upper chamber and 0.6 mL of chemotaxis buffer with or without 100 ng/mL SDF-1 was added to the lower chamber. The chambers were incubated at 37°C in 5% CO_2 for 4 hours, then the upper chamber was carefully removed. Cells that had migrated into the lower chamber were counted using FACScalibur flow cytometry, with appropriate gating, for 20 seconds at a high flow rate. The migration rate was determined by calculating the percentage of input cells migrating into the lower chamber (average events for 20 seconds/ average input cell events for 20 seconds × 100%).

Statistical analysis

Normally distributed data are expressed as means ± standard deviation (SD). Some data are described by both the mean ± SD and median (range). The statistical significance of overall differences among multiple groups was analyzed by the Kruskal-Wallis non-parametric analysis or the ordinary ANOVA test of variance using SPSS 11.0. If the test showed a statistical significance, pairwise comparisons were done using the *multiple-comparisons* criterion. Differences between two independent groups were analyzed by the Mann-Whitney rank sum test, and the paired t test when appropriate. *p* values of less than 0.05 were considered statistically significant.

Results

The expansion efficiency of CD34$^+$ cells

The expansion efficiency was expressed by the fold-expansion of various cell subsets at different time points during culture, where the fold-expansion is the increase of cells with respect to the input cells. Over the whole 2-week culture period, the proportion of CD34$^+$ cells in the expanded products decreased from 98.14% on day 0 to 18.95%, 8.88% and 5.02% on day 7, day 10 and day 14, respectively. In contrast, the absolute number of nucleated cells and CD34$^+$ cells increased and the fold-expansion on day 14 was 894.8±217.5 and 69.7±41.6, respectively (n=11). The numbers of CD34$^+$ cells expressing various adhesion molecules all increased to a great extent, from 15-fold for CD34$^+$CD54$^+$ cells to 72-fold for CD34$^+$CD49e$^+$ cells by day 14 (Figure 1A). CD49d$^+$, CD49e$^+$, CD11a$^+$, and CD44$^+$ subsets of the CD34$^+$ cell population increased throughout the 14-day culture period and reached a maximal amplification on day 14 (n=4).

Expression of adhesion molecules on fresh and re-selected CD34$^+$ cells

The expression of various adhesion molecules on CD34$^+$ cells was measured by mean fluorescence intensity (MFI). Most freshly purified CD34$^+$ cells expressed CD49d (93.4±3.8%), CD49e (94.9±2.7%) and CD44 (96.7±2.7%). However, the MFI of these molecules on CD34$^+$ cells varied from 339.8±130.1 (CD44), 87.2±5.3% (CD11a) to 55.6±12.9% (CD54) (Figure 1B).

The percentages of CD11a$^+$, CD44$^+$ and CD49e$^+$ subsets within the *ex vivo* expanded CD34$^+$ population remained the same as those in freshly isolated UCB CD34$^+$ cells. The proportion of CD49d$^+$ cells increased significantly during the first 10 days of culture ($p<0.05$ on day 7 and day 10) and then declined by day 14 ($p<0.05$ vs day 7 and 10). It was interesting to note that the expansion did not cause any significant change in the MFI of CD11a, CD44 and CD49e (Kruskal-Wallis test, $p>0.05$). However, CD54 expression on expanded CD34$^+$ cells began to decrease slightly on day 7 and was significantly lower on day 10 and day 14 than it was on the fresh CD34$^+$ cells ($p<0.05$ vs day 10 and day 14). The MFI of CD54 expression on CD34$^+$ cells did not change significantly during the 2 week culture (Table 1).

Adhesion of CD34$^+$ cells to fibronectin

Because the expression of the various molecules on CD34$^+$ cells changed to different extents during expansion, we examined the ability of UCB HSPC to adhere to fibronectin. Freshly isolated CD34$^+$ cells adhered to a fibronectin-coated filter. A mean of 28% spontaneous adhesion and was observed. The SDF-1 (100 ng/mL) induced adhesion of CD34$^+$ cells to fibronectin was significantly greater than the spontaneous adhesion at all time points tested ($p<0.05$, SDF-1 induced adhesion rate vs spontaneous adhesion rate, n=3).

Experiments were then performed to examine whether the adhesion activity of CD34$^+$ cells could also be changed during growth factor-activated expansion. Figure 2 shows that at each time point, SDF-1-induced adhesion was higher than spontaneous adhesion ($p<0.05$ on day 7 and day 10, n=3). The adhesion rate increased during the first 10 days of culture: both spontaneous and SDF-1-induced adhesion reached peak levels (62.8±12.8% and 90.5±11.7%, respectively) on day 10 and then declined on day 14.

Migratory capacity of CD34$^+$ cells

Spontaneous and SDF-1-induced migration was analyzed. The capacity of fresh CD34$^+$ cells to migrate differed considerably, with a mean 6.6±2.4% (median, 7.42%, range, 3.9-8.6%) of spontaneous migration and 26.7±22.8% (median, 12.59%, range, 7.3%-59.3%) of SDF-1-induced migration. The presence of SDF-1 (100 ng/mL) in the lower compartment of the

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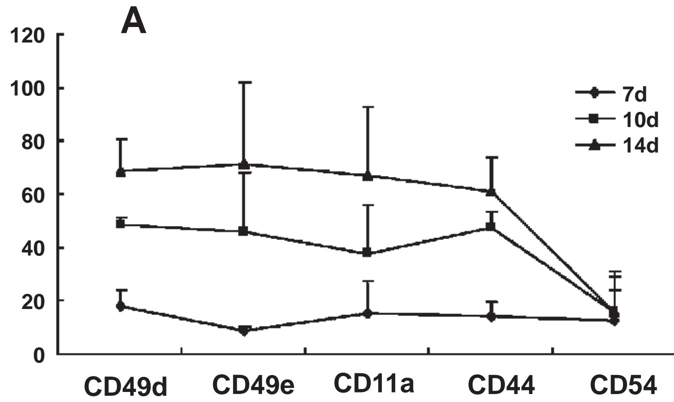

Figure 1. The expansion efficiency of cord blood CD34+ cells. The expansion efficiency is expressed by the fold-expansion of various cell populations expressing different adhesion molecules at each time point during culture. The fold-expansion is the increase of cells with respect to the input cells. A. The fold-expansion of cells expressing CD49d, CD49e, CD44, CD11a and CD54. Data represent the mean ± SD of the determinations from four separate experiments. B. Double-color flow cytometric analysis of the distribution and kinetics of adhesion molecules on CD34+ cells.

B

Day 0 | Day 7 | Day 10 | Day 14

Control

CD49d

CD49e

CD44

CD11a

CD54

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Table 1. The expression of adhesion molecules during expansion.

Day	Expression	CD49d	CD49e	CD11a	CD44	CD54
0	Positive rate (%)	93.36±3.75	94.99±2.74	87.15±5.31	96.74±2.67	55.60±12.93
	MFI	55.27±24.34	54.36±21.16	205.95±83.15	339.78±130.14	44.65±26.97
7	Positive rate (%)	99.59±0.21*	96.47±3.02	99.73±0.15	99.51±0.24	32.58±18.18
	MFI	45.39±13.02	50.17±15.70	272.76±76.65	185.68±14.52	30.82±13.73
10	Positive rate (%)	99.48±0.23*	99.19±0.32	99.84±0.17	99.83±0.05	12.64±6.92*
	MFI	39.57±14.32	55.20±7.93	268.10±19.27	206.51±58.05	21.27±4.05
14	Positive rate (%)	97.62±0.77$^{\$\#}$	95.66±0.55	99.43±0.37	99.74±0.23	9.73±9.30*
	MFI	45.12±15.28	56.80±7.06	259.89±65.48	214.92±30.63	31.70±10.51

*MFI: mean fluorescence intensity; *p<0.05 as compared with fresh purified CD34$^+$ cells; $^{\$}$p<0.01 as compared with CD34$^+$ cells re-selected on day 7; $^{\#}$p<0.05 as compared with CD34$^+$ cells re-selected on day 10.*

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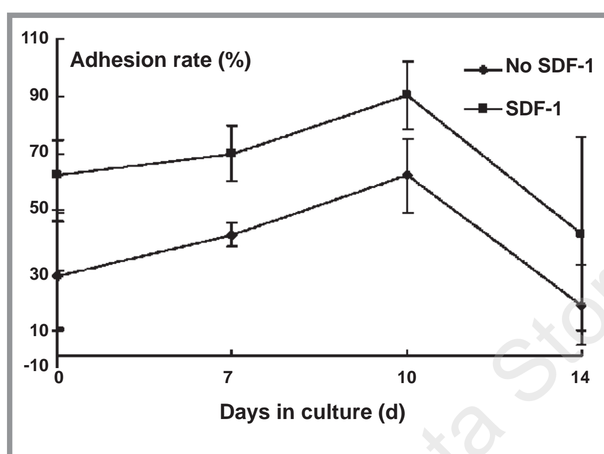

Figure 2. The kinetics of adhesion efficiency during the two-week expansion of cord blood CD34$^+$ cells. Data represent the mean ± SD of the determinations from three separate experiments.

transwell induced a greater migration of both fresh and expanded CB CD34$^+$ cells than that noted for the spontaneous state. Both the spontaneous and SDF-1-induced migration after time was compared to the corresponding baseline migration (p>0.05). Spontaneous migration quickly decreased in the second week (p<0.01 on day 7 and day 14, n=3). On day 14, expanded CD34$^+$ cells showed hardly any spontaneous migration over filters. The SDF-1-induced migration on day 10 was comparable to that on day 0 (p>0.05, n=5), but significantly lower on day 14 (p<0.01, n=5).

CXCR4 expression

The binding of SDF-1 to the receptor CXCR4 was recently found to contribute to the homing of HSPC to bone marrow. To determine whether the difference in SDF-1-induced migratory capacity between the fresh and expanded CD34$^+$ cells was due to the sensitivity of HSPC to SDF-1, CXCR4 expression on CD34$^+$ cells was measured by FACS analysis. The total number of CD34$^+$CXCR4$^+$ cells was significantly increased in the early stage of culture, reached a peak level on day 10 (about 21 times higher than the initial level), and then decreased (Table 2).

The kinetics of CXCR4 expression on CD34$^+$ cells was then investigated. Figure 4 shows that CXCR4 expression on expanded CD34$^+$ cells did not differ significantly from that on fresh CD34$^+$ cells during the first 10 days (p>0.05 on day 7 and day 10, n=3), but that the expression of CXCR4 on the expanded CD34$^+$ population decreased significantly by day 14 (p<0.05, n=3). Nevertheless, the MFI of CXCR4 expression on expanded CD34$^+$ cells at different time points was not significantly different (Table 2) (Kruskal-Wallis Test, p>0.05, n=3).

Discussion

Published data on *ex vivo* expansion of HSPC with repopulating ability are conflicting. In several studies, culture systems containing two of the three early-acting cytokines that we used (FL, SCF and TPO) were described to allow substantial expansion of primitive LTC-IC and CD34$^+$CD38$^–$ subpopulations.[22-25] Piacibello and co-workers[26] described a serum-containing long-term expansion system allowing substantial amplification of CD34$^+$/CD38$^–$ cells and LTC-IC after 20 weeks in the presence of a simple combination of FL and TPO. They subsequently also reported a substantial expansion of SCID-repopulating cells (SRC) in stroma-free suspension cultures of human CD34$^+$ UCB cells in the presence of FL, TPO, SCF and IL-6 for up to

Table 2. The fold-expansion and expression of CXCR4 on UCB CD34⁺ cells.

	Day 0	*Day 7*	*Day 10*	*Day 14*
Fold-expansion of $CD34^+/CXCR4^+$ population	1	9.59±6.53	21.242.91	14.8±1.78
Percentage of positive cells (%)	45.28±19.50	34.53±11.79	21.07±9.91$^{\#}$	9.07±5.96*
Mean fluorescence intensity (MFI)	43.20±37.46	34.34±4.99	34.37±9.27	64.56±8.91$^{\#}$

Compared with the value on day 0, $^{\#}p<0.05$, $^{*}p<0.01$.

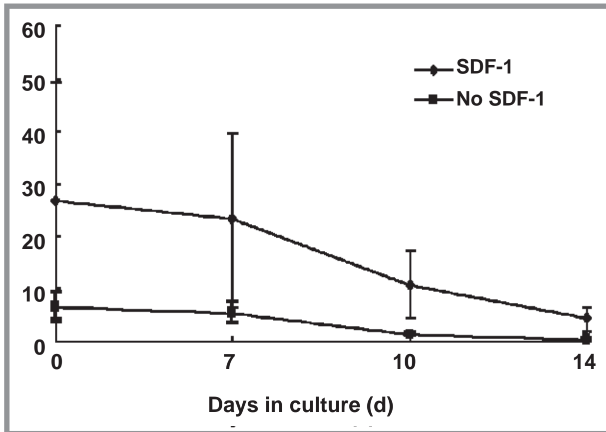

Figure 3. The kinetics of migration efficiency of cord blood $CD34^+$ cells during expansion. $^{\#}p<0.01$ vs day 0 values and $^{*}p<0.05$ vs values of spontaneous state, as determined by Student's t test.

12 weeks.[27] Conversely, Peters' group[28-29] reported that murine hematopoietic stem cells acquired an engraftment defect after *ex vivo* expansion in the presence of IL-3, IL-6, IL-11 and SCF in both normal and irradiated hosts. However, little is known about the homing-related characteristics of HSPC during expansion.

The present study was specifically designed to study the adhesion and migratory characteristics of UCB $CD34^+$ cells during cytokine-mediated *ex vivo* expansion. In accordance with our previous reports,[19] which showed that the number of $CD34^+$ cells increased significantly during the 2-week culture, the present study demonstrated that the numbers of $CD34^+$ cells expressing various adhesion molecules also increased to a great extent. Moreover, a remarkable increase of $CXCR4^+$ $CD34^+$, an important cell population with a homing advantage, was observed. The results obtained from this study once again demonstrate the efficacy of our *ex vivo* culture system in expanding HSPC of various stages.

We found that the expression of CD49d, CD49e, CD11a and CD44 on the $CD34^+$ cells expanded in our culture system was increased or quite similar to that on the fresh UCB $CD34^+$ cells. The MFI of these adhesion molecules on expanded $CD34^+$ cells was not distinct during the culture. Although the expression of CD54 (ICAM-1) on expanded $CD34^+$ cells decreased obviously during the second week of culture, the percentage of $CD54^+$ cells among the expanded $CD34^+$ cells on day 7 was comparable to that at baseline. The MFI of CD54 at different expansion time points did not visibly decrease. Therefore, our short-term expansion system maintained most of the homing-related adhesion molecule expression. These data are consistent with those of Chute,[30] who reported that CD11a and CD44 expressed on the bone marrow $CD34^+$ population was maintained and the expression of CD49d increased after 7-day *ex vivo* expansion. Peled *et al.*[31] demonstrated that during the engraftment of human stem cells in NOD/SCID mice, antibodies to either

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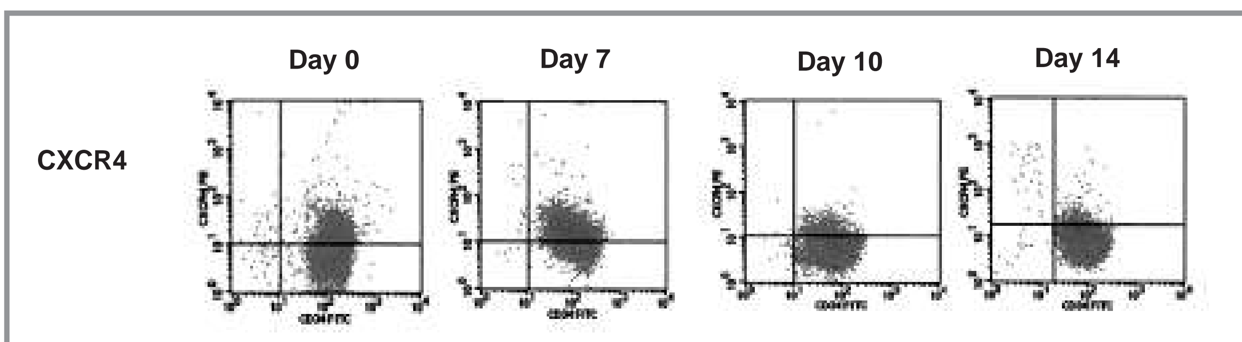

Figure 4. The kinetics change of CXCR4 expression on $CD34^+$ cells.

CD49d or CD49e can prevent engraftment of human CD34$^+$ cells and anti-CD11a antibodies significantly reduced the engraftment levels. Hence, the maintenance of the expression of adhesion molecules on expanded CD34$^+$cells may be crucial to the engraftment potential of expanded HSPC during transplantation.[32]

Given the changes in the various adhesion molecules during the culture, we wanted to know how these changes would influence the homing of the expanded CD34$^+$ cells. We, therefore, re-selected CD34$^+$ cells from the expanded products at different time points, and evaluated the adhesion and migration function of these cells *in vitro* as a surrogate for an *in vivo* homing assay. Our results showed a broad diversity of adhesive and migratory properties of primary CD34$^+$ cells among different individuals, a phenomenon also observed by Voermans *et al*.[33] Interestingly, we found that the levels of both spontaneous and SDF-1-induced migration of expanded CD34$^+$ cells remained similar to the initial levels in the first week, before showing a marked decrease in the day 10 and day 14 cultures. In addition, the adhesion activity of expanded CD34$^+$ cells rose up to day 10 and subsequently decreased by day 14. These data suggest that cytokine activation leads to a specific up-regulation or at least maintains adhesion capacity and transmigration of reselected CD34$^+$ cells for one week. Although extended culture may partly impair the homing potential of expanded CD34$^+$ cells, the addition of PF4, a CXC-chemokine, to the culture blocked the decline in adhesion noted in the cells cultured for 10-14 days (*data not shown*). Therefore, further improvement to the present protocol could help the expanded HSPC to sustain their intrinsic homing potential for even longer.

CXCR4 is the receptor for SDF-1 and appears to play a particularly prominent role in the homing and engraftment of stem cells.[35-36] We did not find distinct changes in CXCR4 expression on cultured CD34$^+$ cells on day 7 and day 10, while a significant decrease was observed on day 14. Nevertheless, given the great expansion of CD34$^+$ cells during the culture, the total number of CD34$^+$ CXCR4$^+$ cells increased markedly in the first 10 days of culture. Furthermore, the MFI of CXCR4 expression on expanded CD34$^+$ cells did not change significantly throughout the two-week period. These data suggest that the transmigrating ability of CD34$^+$ cells expanded for one week was comparable to that of fresh CD34$^+$ cells and that the chemotactic response of CD34$^+$ cells was somehow related to the expression of CXCR4. Our results are in good agreement with those reported by Jo *et al*., who found that cobblestone area-forming cells (CAFC) and LTC-IC expanded in the presence of a KL+TPO+FL cytokine combination for 7 and 14 days did not lose chemotactic responsiveness and that these features were compromised by the addition of IL-3.[37]

In order to evaluate the *in vivo* engraftment capacity of expanded cells derived from an UCB CD34$^+$ population, Zanjani's group[38] transplanted cells cultured using the same culture protocol as ours into fetal sheep. They found that 7-day-expanded cells engrafted primary, secondary, and tertiary recipients while 14-day-expanded cells engrafted primary and to a lesser degree secondary recipients but failed to engraft tertiary recipients. This group subsequently observed the engraftment of the cells expanded with addition of FL, SCF and IL-7 using the same transplant model.[39] Similar results have been described by Romanith *et al*.[40] using a NOD/SCID mice transplanted model. They reported no differences between the recipients of unmanipulated CD34$^+$ UCB cells and those expanded for 7 days in the presence of SCF, TPO, FL, and IL-3. Therefore, CD34$^+$ cells expanded for one week maintained long-term repopulating ability although this capacity decreased during extended culture. These *in vivo* results parallel our *in vitro* observations.

In summary, the present results clearly demonstrate that UCB-derived HSPC can be significantly expanded *ex vivo* in our short-term culture system, consisting of QBSF-60 serum-free medium with a simple combination of early-acting cytokines: SCF, FL and TPO. Most importantly, the expanded HSPC would sustain most of the homing-related characteristics and activity during one week of culture, including expression of adhesion molecules, migration efficiency, and CXCR4 expression. Our culture protocol may pave the way for the *ex vivo* expansion of transplantable human HSPC for clinical applications.

Contributions. QLZ and LQ did the experiments including cell cultures, FACS manipulation, data collection and analysis, and prepared the manuscript. QL and HM did the immunophenotypic characterization of the UCB samples. JH performed the cell cultures. RHH participated the design of ex vivo expansion system of cord blood CD34+ cells. ZC Han was in charge of the design of this study and its interpretation as well as the final preparation of the manuscript. All authors were involved in the revision of the manuscript and have approved the final version of manuscript. The authors reported no potential conflicts of interest.

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