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A human stromal-based serum-free culture system supports the ex vivo expansion/maintenance of bone marrow and cord blood hematopoietic stem/progenitor cells

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***Objective*. We investigated the role of human stromal layers (hu-ST) on the ex vivo expansion/maintenance of human hematopoietic stem/progenitor cells (HSC) from adult bone marrow (BM) and umbilical cord blood (CB).**

***Materials and Methods*. BM and CB CD34$^+$-enriched cells were cultured in serum-free medium supplemented with SCF, bFGF, LIF, and Flt-3, in the presence or absence of stroma, and analyzed for proliferation, phenotype, and clonogenic potential.**

***Results*. Significant expansion of BM and CB CD34$^+$ and CD34$^+$CD38$^-$ cells were achieved in the presence of hu-ST. The differentiative potential of both BM and CB CD34$^+$-enriched cells cocultured with hu-ST was primarily shifted toward the myeloid lineage, while maintaining/expanding a CD7$^+$ population. Clonogenic analysis of the expanded cells showed increases in progenitors of the myeloid lineage, including colony-forming unit-granulocyte, macrophage (CFU-GM) and colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-Mix) for both BM (stroma and stroma-free conditions) and CB cells in the presence of stroma.**

***Conclusions*. These results indicate that adult hu-ST in the presence of appropriate cytokines can be used to efficiently expand/maintain myeloid and lymphoid cell populations from human BM and CB HSC. © 2005 International Society for Experimental Hematology. Published by Elsevier Inc.**

The ability to successfully expand hematopoietic stem cells (HSC) ex vivo would not only have a profound impact in the way that HSC transplantation is thought out, but could also expand the limits of tumor cell purging and somatic cell gene therapy [1–3]. The difficulties associated with inadequate numbers of HSC collected for transplantation from autologous or allogeneic sources such as bone marrow (BM) or umbilical cord blood (CB) would be largely eliminated, and human CB would constitute a reliable and attractive alternative to BM as a source of hematopoietic progenitors [1,4] for patients with malignant and nonmalignant conditions who lack traditional donors [5–7]. A limiting factor for the use of CB is that it contains a finite number of hematopoietic stem and progenitor cells, which may be not enough for the hematopoietic rescue of larger recipients undergoing transplantation, as well as patients with diseases known to be resistant to engraftment, such as Fanconi anemia, chronic myelogenous leukemia, and severe aplastic anemia.

One strategy to increase the cell dose available from a single CB unit is ex vivo expansion. The CB contains a high number of primitive progenitor cells with greater in vitro expansion ability and in vivo engraftment capability when compared with other sources [1]. Furthermore, the reduced alloreactivity of cord blood cells contributes to the lower risk of graft-vs-host disease (GVHD) in patients transplanted with CB when compared to those transplanted with allogeneic adult BM [2,3,5–7]. Although substantial advances have been made in identifying cytokine combinations that are able to maintain or expand to some extent HSC [4, 8–16] or important stromal-derived factors [17,18], an ideal

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system that allows extensive expansion of these cells while maintaining their engraftment capability remains to be defined [1,19]. Some of the most promising results with ex vivo manipulation of human HSC used culture systems in which feeder layers of cells, most of murine origin, were combined with cytokines to achieve long-term ex vivo maintenance and expansion of human HSC while preserving the ability of the manipulated cells to engraft in an in vivo model [1,14,20]. However, murine models have shown an engraftment defect of stimulated HSC by revealing that HSC activation in vitro with various combinations of growth factors results in a decrease of in vivo repopulating ability compared to unmanipulated marrow [21–23]. In some studies, the lack of reconstituting ability of expanded cells was related to the specific cytokine combinations used [24–26], while others have shown the importance of HSC cell-cycle status on the engraftment ability after ex vivo expansion [27,28]. Several groups showed that ex vivo culture systems were able to preserve the ability of the manipulated cells to engraft in an in vivo model [1,5,13,19,29,30]. Concerns have been raised by regulatory agencies worldwide regarding the safety of products, used for the treatment of patients that are produced in culture media containing animal-derived components, such as serum, due to the potential for contamination of infectious agents. Furthermore, the in vitro incubation of an HSC graft with a feeder layer with ill-defined pathogenic potential, such as from a xenogeneic origin and/or modified by viral transformation [31], also poses a risk and is unlikely to find clinical application. Thus the advantages of being able to expand human HSC under serum-free conditions [30] using stromal layers of human origin would have significant clinical applications.

In the present studies, we evaluated the ability of a human stromal-based serum-free culture system (hu-ST) to support the ex vivo expansion/maintenance of human BM and CB HSC. Adult human BM and CB $CD34^+$-enriched cells were cultured in serum-free medium for several days in the presence or absence of allogeneic hu-ST with SCF, bFGF, LIF, and Flt-3, and the cultures were analyzed for expansion, phenotype, and clonogenic ability. This new culture system allowed the successful expansion/maintenance of both myeloid and lymphoid cells from both BM and CB HSC.

Material and methods

Human donor cell preparation

Heparinized human bone marrow was obtained from healthy donors after informed consent. Umbilical cord blood (UCB) samples were obtained from the Pediatric Stem Cell Transplant Program at Duke University Medical Center (Durham, NC, USA). After obtaining maternal donor consent, fresh blood was collected from the umbilical cord vein using the method previously described in the National Heart, Lung and Blood Institute (NHLBI) for Cord Blood Transplantation. The UCB units that did not meet the criteria for storage and banking at Duke Medical Center and were going to be discarded were used in this study. Low-density bone marrow mononuclear cells (BMMNC) or cord blood mononuclear cells (CBMNC) were separated by a Ficoll density gradient (1.077 g/mL) (Sigma, St Louis, MO, USA) and washed twice in Iscove's Modified Dulbecco's Medium (IMDM; Gibco Laboratories, Grand Island, NY, USA). BMMNC and CBMNC from each donor were enriched for $CD34^+$ cells using magnetic cell sorting (Miltenyi Biotec Inc. Auburn, CA, USA).

Human bone marrow stromal cell cultures

Isolated BMMNC obtained from healthy donors were cultured in gelatin-coated T_{25} flasks with Mesenchymal Stem Cell Basal Medium (MSCBM) (Poietics, Cambiex Bioscience, Baltimore, MD, USA). Stromal layers were obtained after culture for 10 days and then γ-irradiated (14 Gy) with a ^{137}Cs source. The irradiated stromal layers were maintained at 37°C under 5% CO_2 humidified air and then used within 1 to 5 days.

Ex vivo expansion of $CD34^+$-enriched cells

BM and CB $CD34^+$-enriched cells (1 to 3×10^6 for BM and 0.75 to 1.5×10^6 for CB) were cultured in T_{25} flasks (5 mL) for several days in QBSF-60 serum-free medium (a kind gift of Dr. Ron Brown, Quality Biological, Gaithersburg, MD, USA) at 37°C under 5% CO_2 humidified air, in the presence or absence of hu-ST with the following cytokines: SCF (100 ng/mL), bFGF (5 ng/mL), LIF (10 U/mL), and Flt-3 (100 ng/mL) (Peprotech, Rocky Hill, NJ, USA). Cultures were half-fed every 2 to 4 days with half of the cultures being harvested and used for analysis, and the same volume being replaced with fresh media.

Proliferative and phenotypic analysis

The ex vivo expansion of the $CD34^+$-enriched populations was determined at each time point by counting the content of hematopoietic cells in each culture flask using trypan blue stain 0.4% solution (GibcoBRL, Grand Island, NY, USA) and analyzed for stem cell and lineage content by flow cytometry (FACScan equipment, Becton-Dickinson, San Jose, CA, USA) using monoclonal antibodies against CD3, CD7, and CD19 to evaluate lymphoid lineage differentiation; CD14, CD15, and CD33 to evaluate myeloid differentiation; and CD34 and CD38 to assess the percentage of stem cells remaining in culture (Becton-Dickinson Immunocytometry Systems [BDIS], San Jose, CA, USA). Flow cytometric analysis was performed at each time point of culture, by incubating harvested cells with different fluorescent conjugated monoclonal antibodies at room temperature for 15 minutes. The cells were then washed in phosphate-buffered saline (PBS) 1% sodium azide (Sigma, St. Louis, MO, USA) and fixed with 2% paraformaldehyde (Sigma). Isotype controls were used in every experiment. A minimum of 10,000 events was collected for each sample.

Clonogenic assays

Assays for clonogenic progenitors were performed in triplicate in MethoCult GF H4434 (Stem Cell Technologies Inc, Vancouver, BC, Canada) with unexpanded $CD34^+$-enriched cells and expanded populations. Cultures were incubated in a humidified incubator at 37°C in 5% CO_2 in air. After 14 days colonies were counted and categorized according to standard criteria [30].

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). Comparisons between experimental results were determined by

two-sided nonpaired Student's *t*-test analysis. A *p* value less than 0.05 was considered statistically significant.

Results

Evaluation of ex vivo expansion of CD34$^+$ -enriched cells in serum-free medium with selected cytokines, in the presence and absence of human stroma

Ex vivo expansion of human CD34$^+$-enriched cells derived from either BM or CB in QBSF-60 serum-free medium supplemented with SCF, bFGF, LIF, and Flt-3 in the presence of stroma was evaluated in comparison to stroma-free BM and CB cultures. Figure 1A and B shows the fold increase in total number of cells with the time in culture obtained when BM and CB CD34$^+$cells were cultured either with or without stroma. In BM cultures the total expansion as assessed by cell number in both stromal and stroma-free system was minimal and similar until day 9 of the culture. However, after this time point, and as can be seen starting at day 14 of culture, CD34$^+$-enriched cells cultured in the presence of stroma began to proliferate more effectively when compared with stroma-free cultures. The difference in proliferation rate was more noticeable in the last days of culture, as can be seen in Figure 1A, in which BM CD34$^+$-enriched cells cultured in the presence of stroma reached a fold increase of 260 (range 145-fold to 375-fold) at day 18 of culture compared with 32-fold in the stroma-free culture system by the same day.

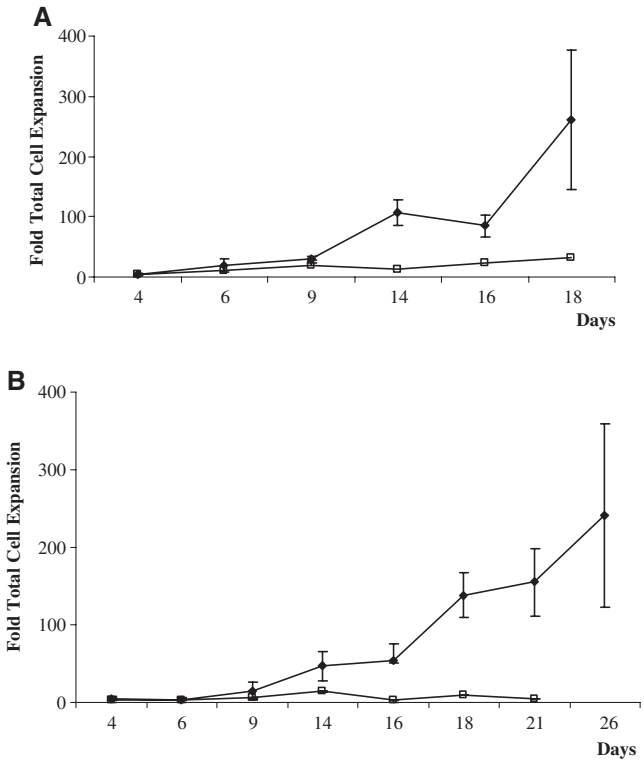

Figure 1. Total cell expansion of CD34$^+$-enriched cells cocultured in the presence/absence of human stromal layers. Cell numbers for hu-ST system (black line, ●) and in the absence of stromal layer (dashed line, □) are expressed as mean fold expansion ± SEM. **(A)**: BM cells (1–2.5 × 10^6) were cultured for 2–3 weeks in the presence (n = 4) or absence (n = 3) of stroma. **(B)**: CB cells (0.1–1.8 × 10^6) were cultured for 3–4 weeks in the presence (n = 4) or absence (n = 3) of stroma. Cell output from both CB and BM cultured in the presence of stroma was significantly higher than that obtained in stroma-free cultures from day 14 until the end of the culture ($p < 0.05$). Because CB cultures continued to proliferate after day 18 of culture we have included time points up to day 26, at which time these cultures reached the maximal output.

As was expected, in the absence of stromal layers, CB cultures were unable to expand (Fig. 1B), since the combination of cytokines selected was anticipated to exert their effect through stromal or accessory cells. In the presence of stromal layers, CB CD34$^+$-enriched cells were able to proliferate to the same extent as their BM CD34$^+$ counterpart grown in the same conditions, although at a later time point. Also, in similarity with BM CD34$^+$-enriched cells cocultured with stromal layers, CB CD34$^+$ cell expansion started to be noticeable at day 14, and at day 18 the total fold increase in CB CD34$^+$ expanded cells was 138 ± 28. The maximal expansion seen with the CB CD34$^+$-enriched cells cocultured with stroma was observed at day 26 with a mean 241-fold increase (range 124-fold to 358-fold). The expansion of both CB and BM CD34$^+$-enriched cultures was directly correlated to the fraction of CD34$^+$ cells in the initial population.

Evaluation of ex vivo expansion of CD34$^+$ and CD34$^+$CD38$^-$ BM and CB cells in the presence and absence of stroma

As can be seen in Figure 2A (BM CD34$^+$ cells) and Figure 2B (BM CD34$^+$CD38$^-$ cells), an increase in absolute numbers of CD34$^+$cells and CD34$^+$CD38$^-$ cells was observed with the time in culture despite the decrease in percentage of these same cell populations (Table 1). During the first four days of culture, the presence/absence of a stromal layer in the cultures did not have a significant impact on the ability of the CD34$^+$-enriched cell population from BM to expand its CD34$^+$ cell and CD34$^+$CD38$^-$ content. However, at days 10 and 14, a significant difference was found in the expansion rate of cells with both CD34$^+$ and CD34$^+$CD38$^-$ phenotypes when the CD34$^+$-enriched cell population from BM were cocultured with stromal layers. On day 14, a 7.0-fold ± 1.9-fold increase in CD34 content and 57.8-fold ± 20.3-fold increase in the CD34$^+$CD38$^-$ compartment was seen (Fig. 2A and B).

As shown in the previous section, CB CD34$^+$-enriched cells were only able to proliferate in the presence of stromal layers. The fold increase in cells with a CD34$^+$ phenotype in CB cultures on day 21 was 13.2 ± 2.2 and at day 26 a 35-fold increase of CD34$^+$ cells was observed (Fig. 2C). The fold increases in CD34$^+$CD38$^-$ cells were 15.9 ± 1.0 and 48, by day 21 and 26, respectively (Fig. 2D).

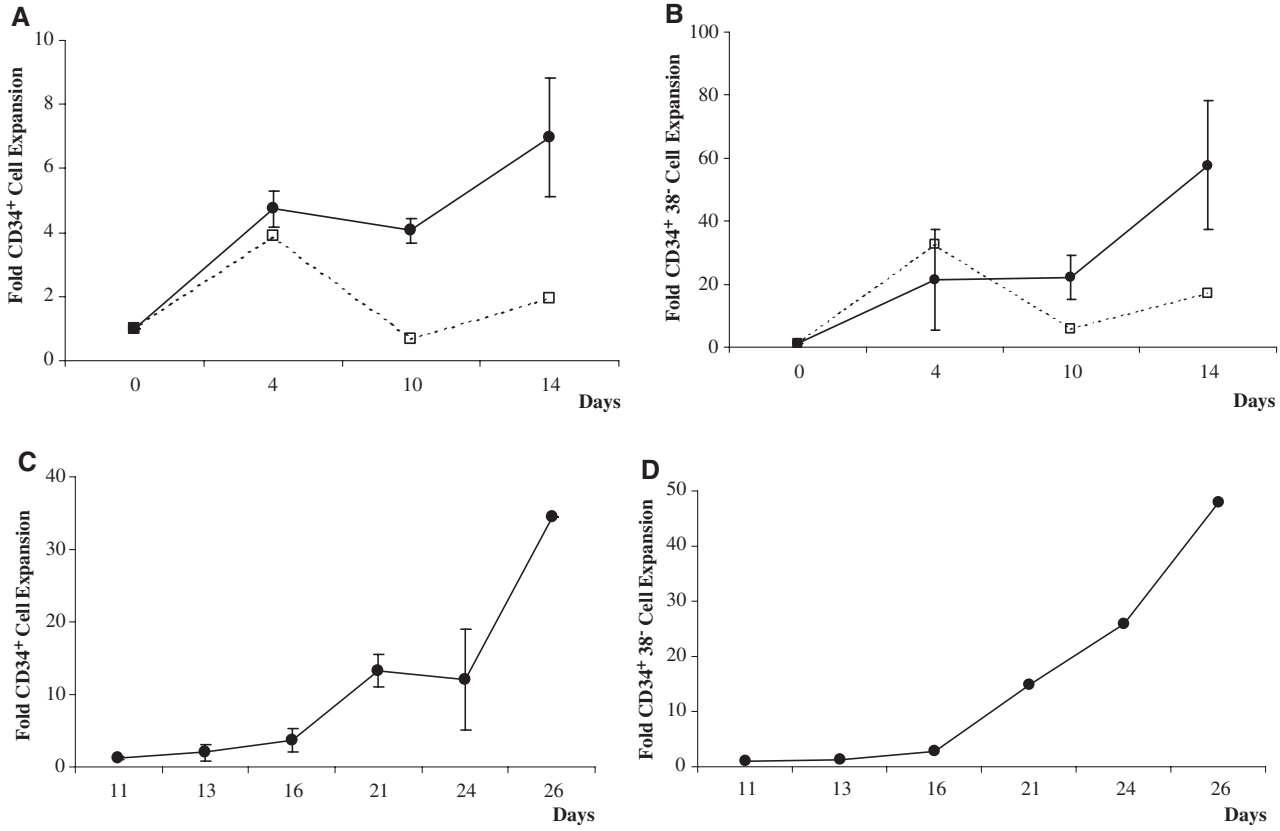

Figure 2. Fold increase in expansion of BM- and CB-derived $CD34^+$ cells in culture. Black line, ● represents fold increase for cells grown in the presence of stroma; dashed line, □ represents the cultures in the absence of stromal layers (data are expressed as mean fold expansion ± SEM). Cultured cells were harvested periodically, enumerated, and stained with CD34-FITC and CD38-PE. **(A)**: Fold increase in BM-derived $CD34^+$ cells when cultured in presence (n = 4) or absence (n = 3) of stromal layers. **(B)**: Fold increase in BM-derived $CD34^+CD38^-$ cells when cultured under the same conditions. **(C)**: Fold increase in CB-derived $CD34^+$ cells when cultured in presence (n = 4) or absence (n = 3) of stromal layers. No earlier time points are shown since in CB cultures only by day 11 were we able to achieve significant $CD34^+$ cell expansion; however, CB $CD34^+$ cell expansion was maintained until later time in culture than their BM counterpart. **(D)**: Fold increase in CB $CD34^+CD38^-$ cells cultured in presence of hu-ST is presented.

Effect of culture conditions on differentiation potential of expanded cells

One of the great advantages of using mobilized peripheral blood (PB) in a transplant setting is that the content of mature cells in the transplantation graft decreases the immunosuppression period, reduces the number of transfusions needed, and leads to earlier patient convalescence. We thus wished to evaluate the effect of the culture conditions and expansion

Table 1. Differentiative potential of BM expanded cells

%	$CD34^+$		$CD34^+CD38^-$		$CD15^+$		$CD33^+$		$CD7^+$		$CD14^+$	
Day	w/ st	w/o st	w/ st	w/o st	w/ st	w/o st	w/ st	w/o st	w/ st	w/o st	w/ st	w/o st
0	75.7		8.2		7.6		46.0		9.4		3.5	
4	47.0	49.4	36.0	44.6	54.9	50.9	35.0	52.5	42.3	49.3	10.6	22.3
10	11.8	4.6	10.1	4.5	42.0	30.8	30.8	46.9	65.2	27.4	37.2	25.1
14	6.6	11.0	3.0	10.6	57.2	34.7	30.0	71.4	29.6	27.8	19.0	29.3
# cells ($\times 10^6$)												
0	1.3		0.1		0.1		0.8		0.2		0.1	
4	7.1	5.2	5.4	4.7	8.3	5.4	5.3	5.5	6.4	5.2	1.6	2.4
10	4.9	0.9	4.2	0.9	18.0	5.9	13.0	9.0	28.0	5.3	16.0	4.8
14	12.0	2.6	5.4	2.5	100.0	8.2	54.0	17.0	53.0	5.4	34.0	6.9

BM $CD34^+$-enriched cells were cultured for 2–3 weeks in the presence (w/ st)/absence (w/o st) of hu-ST. Nonadherent cells were harvested periodically and analyzed by flow cytometry. Shown are the results from one representative experiment. The % of cells is represented on top and the absolute number of cells is indicated on the bottom.

on the differentiation potential of the $CD34^+$-enriched cells from BM and CB. To this end, cultures derived from the $CD34^+$-enriched cells either from BM or CB were analyzed for the presence of CD3, CD7, CD14, CD15, CD19, and CD33 by flow cytometric analysis. The results of the differentiative potential of the expanded BM and CB cell populations are presented in Table 1 for BM cells cultured with and without human stroma, and in Table 2 for CB cells expanded in the presence of stroma.

The differentiative potential of both BM and CB cells cocultured with hu-ST was primarily shifted towards the myeloid lineage, with the presence of $CD14^+$, $CD15^+$, and $CD33^+$ cells. BM $CD34^+$-enriched cells cultured in the absence of stroma also differentiated towards a myeloid phenotype, but the percentage of CD15 achieved in the absence of stroma was considerably lower than that obtained in the coculture system. No $CD3^+$ or $CD19^+$ cell populations were detected upon days 6 to 8 in either culture system (data not shown).

Of importance was a significant increase in the percentage of $CD7^+$ cells with time in culture of both BM and CB cells in the cultures grown on stromal layers. In the case of the BM cells, by day 10 the percentage of $CD7^+$ cells was 52.0% ± 14.2%, while with CB cultures, 65.5% ± 3.3% of the cells were $CD7^+$ at day 21, showing that in our culture system we were also able to expand cells with an early lymphocytic phenotype (Fig. 3A and B).

Clonogenic potential of the expanded cells

In order to assess the effects of expansion on the clonogenic potential of $CD34^+$-enriched cells, cells were harvested from the cultures at different time points and 10^3 cells (day 0) or 10^5 cells (other days of culture) were plated in methylcellulose as described in the Material and Methods section. The total fold increase in clonogenic potential was calculated by dividing the number of CFU-Mix, BFU-E, and CFU-GM obtained per 10^3 cultured cells at the different time points by the number of the different colonies obtained at day 0 and multiplying by the fold increase in total number of cells. The total fold increase in clonogenic potential (CFU-Mix, BFU-E, and CFU-GM) at day 14 was 37.9 ± 0.24 for the BM $CD34^+$ cultured in the presence of stroma and 11.4 ± 0.5 for the cultures in the absence of stroma, showing that the cultures in the presence of stroma were able to maintain a higher clonogenic potential after 2 weeks of expansion. Table 3 displays the clonogenic potential with time in culture of BM cells cultured in the presence and absence of hu-ST.

The total fold increase in clonogenic potential in CB cells grown over stromal layers at day 24 of culture was 137.46 ± 2.2 times that of the initial culture. Table 4 displays the clonogenic potential with time in culture of CB cells cultured in the presence of hu-ST.

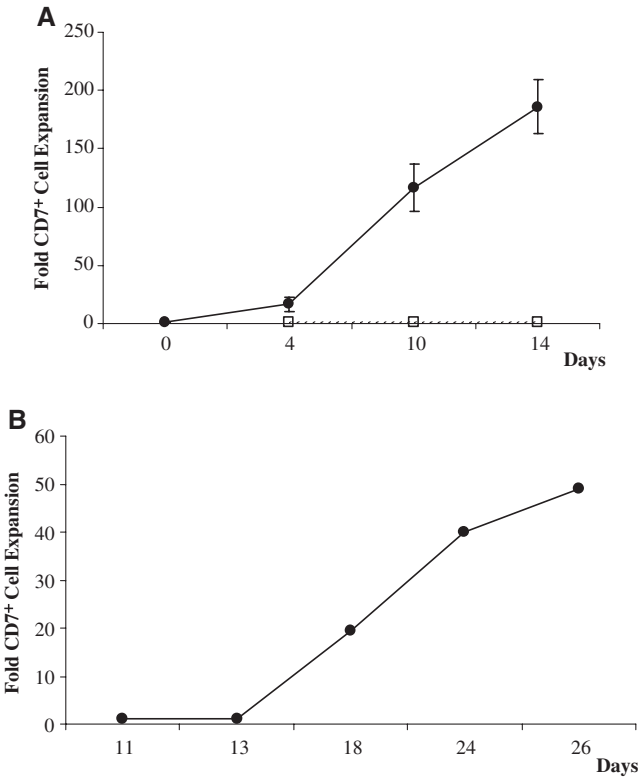

Figure 3. Expansion of $CD7^+$ cells cocultured in the presence/absence of human stromal layers. Cell numbers for hu-ST system (black line, ●) and in the absence of stromal layer (dashed line, □) are expressed as mean fold expansion ± SEM. Cultured cells were harvested periodically, enumerated, and stained with CD7-FITC. **(A)**: Fold increase in $CD7^+$ cells in BM cultures in presence/absence of hu-ST. **(B)**: Fold increase in CB $CD7^+$ cells cultured in presence of hu-ST is presented (one representative experiment); once more, only by day 11 were we able to achieve significant cell numbers to analyze these c.

Table 2. Differentiative potential of CB expanded cells cultured in hu-ST system

% Day	$CD34^+$	$CD34^+CD38^-$	$CD15^+$	$CD33^+$	$CD7^+$	$CD14^+$
11	12.5	9.0	24.5	47.1	51.8	18.2
13	7.2	7.0	24.6	39.3	34.2	13.1
16	7.0	6.7	34.5	27.4	59.7	26.3
21	4.9	4.8	36.5	51.3	62.2	28.0
24	8.7	8.6	29.0	44.3	76.0	49.6
26	0.9	0.9	22.5	57.5	75.4	62.8
# cells ($\times 10^6$)						
11	1.0	0.7	2.0	7.3	4.1	1.3
13	0.9	0.9	3.2	5.0	4.4	1.5
16	2.1	2.0	10.0	8.1	18.0	3.7
21	11.0	11.0	82.0	120.0	140.0	60.0
24	19.0	19.0	63.0	96.0	170.0	110.0
26	24.0	24.0	88.0	150.0	210.0	160.0

CB $CD34^+$-enriched cells were cultured for 3–4 weeks in the presence of hu-ST. Nonadherent cells were harvested periodically and analyzed by flow cytometry. Shown are the results from one representative experiment. The % of cells is represented on top and the absolute number of cells is indicated on the bottom.

Table 3. Clonogenic potential of BM cells cultured in the presence and absence of human stroma

	CFU-Mix		BFU-E		CFU-GM	
Day	w/ st	w/o st	w/ st	w/o st	w/ st	w/o st
0	3		4		11	
4	17 ± 3	18 ± 9	6 ± 3	0	166 ± 23	207 ± 38
7	18 ± 2	NA	0	NA	292 ± 15	NA
10	0	8 ± 2	0	0	296 ± 31	208 ± 3
14	5 ± 1	47 ± 10	6 ± 1	9 ± 7	202 ± 14	392 ± 58

Numbers of colonies obtained per 100,000 cells.
NA: data not available (not enough cells obtained to perform clonogenic assays).

Discussion

A major focus in experimental hematology is the delineation of conditions that would allow HSC to be manipulated in vitro in such a way that they could expand in number yet maintain all of the characteristics that define an HSC. The definition of such strategies would impact profoundly on both clinical HSC transplantation and gene therapy. Thus far, the majority of studies that have demonstrated expansion of long-term engrafting HSC have accomplished this by employing culture systems that combine cytokine stimulation with support of feeder cell layers. Most of the feeder layers that have thus far been screened for the ability to support/expand hematopoietic stem cells are either murine [1,12–14,20,29] or porcine [32–35] in origin. Furthermore, some of these stromal layers require serum for their maintenance and have also been immortalized in order to be able to be propagated indefinitely [31]. The advantages of being able to expand human HSC under serum-free conditions using stromal layers of human origin that could be harvested either from the patient or from allogeneic sources, and that have not been subjected to viral transformation, would have significant clinical applications, since it would reduce the potential for contamination of infectious agents.

In this study we investigated the ability of human stromal layers obtained from normal randomly selected marrow donors, in the presence of SCF, LIF, bFGF, and Flt-3, to support the ex vivo expansion/maintenance of human BM- and CB-derived HSC. Our cocktail of cytokines was anticipated to exert its effect through stromal and/or accessory cells and was chosen to ensure that all stromal layers would support hematopoiesis, and, furthermore, that the process of expansion would not lead to the exhaustion of the more primitive stem cells. LIF is a pleiotropic cytokine identified by its growth and differentiation activities on hematopoietic cells [15]. Shih et al. [12] reported its stromal cell–derived activity that promoted ex vivo expansion of human hematopoietic stem cells, showing that LIF action on HSC is indirect and mediated by stromal cells, leading to an upregulation of a large number of known cytokines and a downregulation of many others. Here, bFGF was used to support growth and maintenance of the stromal feeder layers in the absence of serum [11]. Flt-3 and SCF were included since they are known to positively influence self-renewal, proliferation, and preservation from apoptosis of the more primitive hematopoietic cells, especially in synergy with several other cytokines [1,13]. However, studies using more cytokines to our initial cocktail such as TPO, IL-3, and GM-CSF, singly or in combination, are being performed to investigate whether we can successfully further increase the rates of expansion of $CD34^+$ cells without losing the lymphocytic potential in the expanded cultures.

Our studies used a population of adult BM and CB cells enriched for the surface marker CD34. It could be argued that this population of cells is very heterogeneous and does contain cells that have already committed to various lineages. However, we reasoned that since the majority of transplants performed today using enriched hematopoietic progenitor cells are performed using $CD34^+$-enriched cells, the derivation of methods for expanding the number of long-term engrafting cells within this population would be of direct clinical utility. In our studies we were able to successfully expand BM $CD34^+$-enriched cells up to 260-fold (range 145-fold to 375-fold) and CB $CD34^+$-enriched cells up to 241-fold (range 124-fold to 358-fold). Our observations lead us to conclude that expansion of both CB and BM $CD34^+$-enriched cells is correlated to the initial fraction of $CD34^+$ cells, in such a way that a higher starting number of $CD34^+$ cells leads to greater total cell expansion. We were also able to expand the more primitive $CD34^+CD38^-$ cells and achieved a 57.8-fold ± 20.3-fold increase in this population in BM cultures and a 48-fold increase in CB cultures. We were also able to maintain/expand the clonogenic potential of both HSC populations in the stromal culture system described. The total fold increase in clonogenic potential at two weeks was 37.9 ± 0.24 for the BM $CD34^+$ cultured in the presence of stroma and 137.46 ± 2.2 for the CB cultures at day 24. Other studies by McNiece et al., Berstein et al., and Haylock et al. [35–37] using different cytokine combinations reported fold increases in clonogenic potential of BM expanded cells of 7, 10, and 66, respectively. And Briddel et al. [38] reported in expanded cord blood a 73-fold increase in the clonogenic potential. The cells produced under the

Table 4. Clonogenic potential of CB cells cultured in the presence of human stroma

Day	CFU-Mix	BFU-E	CFU-GM
1	3	7	8
6	65 ± 6	4 ± 1	296 ± 12
8	20 ± 6	0	463 ± 46
11	15 ± 8	0	218 ± 12
13	8 ± 1	6 ± 2	121 ± 17
16	17 ± 5	0	247 ± 26
21	14 ± 2	0	221 ± 21
24	4 ± 2	0	197 ± 9

Numbers of colonies obtained per 100,000 cells.

conditions mentioned in these studies were shifted towards the myeloid lineage. In our culture system, although the differentiative potential of both BM and CB cells cocultured with hu-ST was primarily shifted towards the myeloid lineage with no $CD3^+$ and $CD19^+$ cell populations detected upon days 6 to 8 of culture, both BM and CB cells showed an increase in the percentage of $CD7^+$ cells with time in culture; by day 10 the percentage of BM $CD7^+$ cells was 52.0% ± 14.2% while in CB at day 21, the percentage of $CD7^+$ cells was 65.5% ± 3.3%. Thus, we conclude that our culture system is also able to expand cells with an early lymphocytic phenotype. CD7 is the earliest antigen marker expressed in the T lineage, being found on T cell precursors in fetal liver and thorax prior to thymic colonization, and in thymus and BM [39–42]. Additional experiments are being conducted at this point to address the further differentiative potential of this population. However, preliminary data show the ability of the CD7 population obtained in our culture system to differentiate into NK and dendritic cells [40]. Also, in vivo studies in the fetal sheep model of human hematopoiesis are being performed to address the question of whether the expansion system described herein will allow normal engraftment and differentiation of the expanded cell populations.

In conclusion, our present work indicates that a human stromal-based, serum-free culture system can effectively expand HSC from both BM and CB, while preserving and expanding both myeloid and lymphoid progenitors.

Acknowledgments

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