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Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults

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ABSTRACT **We estimated whether single collections of cord blood contained sufficient cells for hematopoietic engraftment of adults by evaluating numbers of cord blood and adult bone marrow myeloid progenitor cells (MPCs) as detected *in vitro* with steel factor (SLF) and hematopoietic colony-stimulating factors (CSFs). SLF plus granulocyte–macrophage (GM)-CSF detected 8- to 11-fold more cord blood GM progenitors [colony-forming units (CFU)-GM] than cells stimulated with GM-CSF or 5637 conditioned medium (CM), growth factors previously used to estimate cord blood CFU-GM numbers. SLF plus erythropoietin (Epo) plus interleukin 3 (IL-3) enhanced detection of cord blood multipotential (CFU-GEMM) progenitors 15-fold compared to stimulation with Epo plus IL-3. Under the same conditions, bone marrow CFU-GM and CFU-GEMM were only enhanced in detection 2- to 4- and 6- to 8-fold. Increased detection of cord blood CFU-GEMM correlated directly with decreased detection of cord blood erythroid burst-forming units (BFU-E). In contrast, adult bone marrow CFU-GEMM and BFU-E numbers were both enhanced by SLF plus Epo plus IL-3. This suggests that most cord blood BFU-E may actually be CFU-GEMM. Cord blood collections ($n = 17$) contained numbers of MPCs (especially CFU-GM) similar to the number found in nine autologous bone marrow collections. To assess additional sources of MPCs, the peripheral blood of 1-day-old infants was assessed. However, average concentrations of MPCs circulating in these infants were only 30–46% that in their cord blood. Expansion of cord blood MPCs was also evaluated. Incubation of cord blood cells for 7 days with SLF resulted in 7.9-, 2.2-, and 2.7-fold increases in numbers of CFU-GM, BFU-E, and CFU-GEMM compared to starting numbers; addition of a CSF with SLF resulted in even greater expansion of MPCs. The results suggest that cord blood contains a larger number of early profile MPCs than previously recognized and that there are probably sufficient numbers of cells in a single cord blood collection to engraft an adult. Although the expansion data must be considered with caution, as human marrow repopulating cells cannot be assessed directly, *in vitro* expansion of cord blood stem and progenitor cells may be feasible for clinical transplantation.**

Circulating blood cells are derived from hematopoietic stem and progenitor cells (1). Bone marrow is the main source of stem and progenitor cells in the adult, but, ontologically, these cells are found first in yolk sac, next in fetal liver and spleen, and subsequently in fetal bone marrow (1–3). Human umbilical cord blood is a rich source of these parent cells (reviewed in refs. 4 and 5). Our previous study (4) inferred that cord blood from single collections should contain enough stem and progenitor cells for hematopoietic reconstitution in a transplant setting, a possibility verified by successful hematopoietic engraftment of children with human leukocyte antigen (HLA)-matched sibling cord blood cells (5–7).

The critical question addressed here is whether a single collection of cord blood also contains sufficient numbers of immature cells to engraft the hematopoietic system of an adult. We used steel factor (SLF) (reviewed in ref. 8), also termed mast cell growth factor (9), stem cell factor (10), and *kit* ligand (11), alone and in combination with hematopoietic colony-stimulating factors (CSFs), to compare numbers and expansion potential *in vitro* of cord blood and adult bone marrow myeloid progenitor cells (MPCs). SLF detects early populations of granulocyte–macrophage (CFU-GM), erythroid (BFU-E), and multipotential (CFU-GEMM) bone marrow progenitor cells (9–13).

MATERIALS AND METHODS

Cells. Cord and placental blood were obtained as described (4). The Institutional Review Boards ruled these collections to be exempt from the consent process. Blood (≈1 ml) was obtained from infants 1 day after birth as approved by the Institutional Review Board of St. Jude Children's Research Hospital. Human bone marrow cells were obtained from healthy donors who had given informed consent or from unused aliquots of cyropreserved bone marrow harvested for autologous bone marrow transplantation as described elsewhere (14). Cells were left unseparated, or low-density (<1.077 g/ml) cells were retrieved after separation on Ficoll/Hypaque (Pharmacia). Cells were cryopreserved using a liquid nitrogen programmed freezer (Cryomed, Mt. Clemens, MI) (4, 15).

Growth Factors (Cytokines). Purified recombinant (r) murine (mu) and human (hu) SLF (16, 17), hu GM-CSF, hu G-CSF, and hu interleukin 3 (IL-3) were gifts from Immunex (Seattle). Both mu and hu SLF were used at 50 ng/ml (12); hu GM-CSF, G-CSF, and IL-3 were each used at 100 units/ml. Purified rhu erythropoietin (Epo), purchased from Amgen Biologicals, was used at 1 unit/ml. Medium conditioned by the human urinary bladder carcinoma cell line 5637

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Abbreviations: SLF, steel factor; CSF, colony-stimulating factor; MPC, myeloid progenitor cell; GM, granulocyte–macrophage; CFU, colony-forming unit(s); BFU-E, burst-forming unit(s), erythroid; GEMM, granulocyte, erythroid, monocyte, megakaryocyte; r, recombinant; mu, murine; hu, human; IL-3, interleukin 3; Epo, erythropoietin.

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(5637 CM) (18) was used at 10% (vol/vol). Maximally effective concentrations of each cytokine were used.

Colony Assays. The CFU-GM assay (0.3% agar culture medium) and the BFU-E/CFU-GEMM assay (0.9% methylcellulose culture medium) were done as described (4). Cells were plated at 0.25, 0.5, and/or 1.0×10^5 cells per ml and incubated in 5% CO_2 in lowered (5%) O_2 in a humidified atmosphere for 14 days.

Suspension Culture Assay. Cells (4×10^6) were placed in 2 ml of Iscove's modified Dulbecco's medium plus 10% (vol/vol) prescreened heat-inactivated (56°C for 0.5 hr) fetal bovine serum (HyClone) in the absence or presence of cytokines for 7 days under the same incubation conditions as for the colony assays. After 7 days, cells were removed, washed twice, counted, and plated in colony assays.

RESULTS

Enhanced Detection of MPCs in Cord Blood Compared with Adult Bone Marrow. It has become apparent that the sources of stimulation used in our original study (4) underestimated numbers of MPCs in bone marrow, and SLF in combination with these cytokines enhances detection of marrow MPCs (9–12).

The first objective was therefore to reevaluate cord blood MPCs (4, 19) and to compare this with adult bone marrow MPCs (Table 1). SLF plus GM-CSF detected 8- to 11-fold more cord blood CFU-GM than that noted when cells were stimulated with either 5637 CM or GM-CSF. SLF plus Epo detected 11-fold more CFU-GEMM than Epo plus either 5637 CM or IL-3; this reached 15-fold enhancement in detection when IL-3 was added with SLF and Epo. Enhanced detection of cord blood CFU-GEMM directly correlated with decreased detection of BFU-E, an effect not noted with bone marrow, where BFU-E and CFU-GEMM numbers were increased by addition of SLF to culture (Table 1). Addition of SLF with other cytokines to bone marrow cells also detected greater numbers of CFU-GM (2- to 4-fold) and CFU-GEMM (6- to 8-fold), but these increases were not as great as that found with cord blood CFU-GM and CFU-GEMM (Table 1). As reported for human marrow MPCs (9–12), addition of SLF to cord blood and bone marrow cultures with CSFs enhanced colony size 3- to 10-fold compared with no SLF.

Comparison of MPC Numbers in Cord Blood with That Used for Autologous Bone Marrow Transplantation. MPC numbers (specifically, CFU-GM) have estimated the repopulating capacity of autologous and allogeneic adult bone marrow cells (20–23). We thus compared numbers of MPCs in single cord blood collections, with and without SLF, with that in bone marrow transplant samples (Table 2). In single collections of fresh cord blood, there were 45.3–91.6% of the CFU-GM, 29.0% of the BFU-E, and 23.5–24.3% of the CFU-GEMM numbers present in the successful marrow autografts. Thawing of cord blood cells stored frozen for 2 months to 5 years yielded >90% and >80% recoveries of

Table 1. Comparative changes in detection of MPCs in human umbilical cord blood versus adult bone marrow when cells are plated in the presence of r SLF (a c-*kit* ligand)

	Change, -fold					
	Umbilical cord blood			Adult bone marrow		
	Mean ± 1 SEM	Range	No. of samples	Mean ± 1 SEM	Range	No. of samples
CFU-GM colonies with GM-CSF + SLF						
Compared with 5637 CM	+8.2 ± 1.0	+3.5 to +14.5	23	+2.1 ± 0.2	+1.9 to + 2.7	4
Compared with GM-CSF	+10.7 ± 1.2	+2.3 to +30.0	36	+3.6 ± 0.3	+1.9 to + 5.9	28
CFU-GEMM colonies with Epo + SLF						
Compared with Epo + 5637 CM	+11.3 ± 1.2	+2.0 to +24.0	23	ND		
Compared with Epo + IL-3	+10.5 ± 1.1	+2.0 to +30.0	36	+7.9 ± 1.1	+2.7 to +23.0	25
CFU-GEMM colonies with Epo + SLF + IL-3						
Compared with Epo + 5637 CM	+14.7 ± 1.6	+2.0 to +36.0	23	ND		
Compared with Epo + IL-3	+15.1 ± 1.5	+2.0 to +37.0	36	+5.6 ± 0.6	+3.3 to + 7.8	8
BFU-E colonies with Epo + SLF						
Compared with Epo	−7.4 ± 1.2*	1.0 to −16.0	35	+3.2 ± 0.4	1.0 to + 7.4	24
Compared with Epo + 5637 CM	−11.5 ± 2.4†	−1.3 to −33.0	23	ND		
Compared with Epo + IL-3	−15.8 ± 2.6‡	−1.5 to −58.0	35	+1.7 ± 0.8	1.0 to + 6.8	24
BFU-E colonies with Epo + SLF + IL-3						
Compared with Epo	−8.8 ± 1.3§	1.0 to −30.0	35	+3.4 ± 0.6	1.0 to + 3.9	8
Compared with Epo + 5637 CM	−13.0 ± 2.4¶	−1.3 to −47.0	23	ND		
Compared with Epo + IL-3	−18.2 ± 2.8‖	−1.5 to −58.0	35	+1.6 ± 0.2	+1.3 to + 3.8	8

Growth factors were rhu GM-CSF (100 units/ml), rhu or rmu SLF (50 ng/ml), 5637 CM (10%; vol/vol), rhu Epo (1 unit/ml), and rhu IL-3 (100 units/ml). Cord blood results were from a total of 36 different collections, which included defrosts of four cord bloods stored frozen as low-density cells for 5 years and then defrosted, 11 collections stored frozen in unseparated form for from 3 to 26 months and assessed in unseparated form, and 21 freshly obtained collections assessed in unseparated form. Bone marrow results were from a total of 28 different individuals, which included 21 normal healthy donors and 7 patients with ovarian cancer off chemotherapy for 6 months before the study and with normal blood cell counts and no tumor infiltration of the marrow. Control colony numbers for cord blood vs. marrow CFU-GM ranged, respectively, from 5 to 66 and 41 to 112 with 5637 CM and from 4 to 56 and 10 to 72 with GM-CSF. Control colony numbers for cord blood vs. marrow CFU-GEMM ranged, respectively, from 1 to 13 and ND (not done) with Epo + 5637 CM and from 1 to 13 and 1 to 13 with Epo + IL-3. Control colony numbers for cord blood vs. marrow BFU-E ranged, respectively, from 6 to 64 and 16 to 80 with Epo, from 9 to 70 and ND with Epo + 5637 CM, and from 11 to 111 and 42 to 126 with Epo + IL-3.

*Decrease of 71% ± 5%.
†Decrease of 78% ± 4%.
‡Decrease of 85% ± 3%.
§Decrease of 71% ± 5%.
¶Decrease of 82% ± 4%.
‖Decrease of 88% ± 2%.

Table 2. Total number of nucleated cells and myeloid progenitors in single collections of human umbilical cord blood compared with that used for autologous bone marrow transplantation

Cell population	Stimulation *in vitro*	Cord blood (n = 17)		Bone marrow (n = 9)		Cells in cord blood vs. bone marrow, %
		Mean ± 1 SEM	Range	Mean ± 1 SEM	Range	
Nucleated cells × 10^{-9}		1.8 ± 0.2	0.7–4.2	12.8 ± 1.5	6.3–19.4	14.1 (12.7)
Myeloid progenitors × 10^{-5}						
CFU-GM colonies	GM-CSF	4.4 ± 0.8	0.2–10.9	4.8 ± 1.7	0.9–15.5	91.6 (73.3)
CFU-GM colonies + clusters	GM-CSF	14.8 ± 2.3	4.0–33.5	27.9 ± 7.6	2.8–65.4	53.0 (42.4)
CFU-GM colonies	GM-CSF + SLF	9.8 ± 1.8	0.9–27.0	12.5 ± 3.5	3.4–29.4	78.4 (62.7)
CFU-GM colonies + cluster	GM-CSF + SLF	21.5 ± 3.1	5.4–59.2	47.5 ± 12.5	6.9–117.6	45.3 (36.2)
BFU-E colonies	Epo + IL-3	13.1 ± 1.6	6.0–33.6	45.1 ± 11.9	11.1–107.7	29.0 (23.2)
CFU-GEMM colonies	Epo + SLF	10.1 ± 1.9	3.2–22.8	43.0 ± 12.0	11.8–130.4	23.5 (18.8)
CFU-GEMM colonies	Epo + SLF + IL-3	14.0 ± 2.4	4.2–44.2	57.6 ± 15.1	16.4–157.5	24.3 (19.4)

Growth factors were GM-CSF (100 units/ml), rhu or rmu SLF (50 ng/ml), Epo (1 unit/ml), and IL-3 (100 units/ml). Cord blood results are from freshly isolated collections. Cryopreserved autologous bone marrow was transfused into nine patients 23–42 years old (testis cancer, 3; mediastinal germ cell tumor, 1; breast cancer, 1; lymphoblastic lymphoma, 1; large cell lymphoma, 1; Hodgkin disease, 1; mixed cell lymphoma, 1). Eight of nine engrafted (absolute neutrophilic granulocytes, >500 by day +11 to +35). The weights of the recipients averaged 72 ± 4 kg, ranging from 60 to 100 kg. Bone marrow values are from defrosts of cryopreserved cells. Values in parentheses represent those based on comparing cord blood numbers recalculated based on previous information demonstrating respective recoveries of >90% and >80% (n = 10 experiments) for nucleated cells and MPCs from thawed cord blood.

nucleated cells and MPCs (n = 10 samples; data not shown). Taking these recoveries into consideration, single collections of cord blood would still contain 36.2–73.3% of the CFU-GM, 23.2% of the BFU-E, and 18.8–19.4% of the CFU-GEMM that were present in the marrow autografts.

Considerable overlap was seen in MPC numbers found in single cord blood collections compared with those present in the individual marrow collections actually used for transplantation (see range of values in Table 2). Numbers of CFU-GM from donor inoculum have best correlated with bone marrow engraftment in humans (20–23) and with speed of engraftment in mice (24) and humans (25). All 17 cord bloods contained numbers of CFU-GM colonies and clusters recognized by stimulation with GM-CSF and 16 of 17 cord bloods contained numbers of CFU-GM colonies and clusters recognized by stimulation with GM-CSF plus SLF that were higher than that present in the successful marrow autograft with the lowest numbers of these cells.

Concentration of MPCs in Blood of 1-Day-Old Children Compared with Cord Blood. It has been noted that MPC concentration is elevated in blood of newborn children for weeks after birth compared to adult blood (26, 27). However, it was not reported how quickly MPC levels per ml decreased after birth. While the concentration of nucleated cells increased by 24–30 hr after birth, MPCs at this time averaged 30–46% of that found in the cord blood (Table 3).

Expansion of Cord Blood and Bone Marrow MPCs in Short-Term Suspension Cultures. The last objective was to determine whether cord blood MPC numbers could be expanded *in vitro*. Short-term culture of human marrow in the presence of different cytokines has, to a limited degree, allowed for expansion of MPCs (28), and SLF has, in combination with other cytokines, enhanced murine (29–31) and human (32) bone marrow MPC expansion. We compared the capacity of SLF, alone and in combination with different CSFs, to expand MPC numbers of cord blood; this was compared with that noted for adult bone marrow MPCs (Table 4). Incubation of low-density cord blood cells in suspension culture with SLF for 7 days resulted in average increases for CFU-GM, BFU-E, and CFU-GEMM of 7.9-, 2.2-, and 2.7-fold, respectively, compared to starting concentrations of these cells. SLF plus either IL-3, GM-CSF, G-CSF, or Epo resulted in even greater expansion of MPC numbers. The results reflect the average-fold expansion noted with SLF plus a second cytokine that gave the largest expansion of MPCs within an individual experiment. Expansion with a cytokine other than SLF, when this cytokine was used alone, was always lower than with SLF alone. Similar results were noted with bone marrow MPCs (Table 4).

Expansion of MPCs was also noted for unseparated cord blood in two experiments. In one representative experiment, numbers of CFU-GM colonies per ml of cord blood at time 0 were 480 ± 176 (mean ± 1 SD). After 7 days in suspension culture, CFU-GM numbers were 665 ± 86 (no added cytokine), 4268 ± 408 (50 ng of rhu SLF per ml), 8938 ± 340 (SLF plus 100 units of rhu GM-CSF per ml), and 5267 ± 310 (SLF plus 100 units of rhu IL-3 per ml). These were increases compared to day 0 counts of 1.4- (no cytokine), 8.9- (SLF), 18.6- (SLF + GM-CSF), and 11.0-fold (SLF + IL-3). In the two experiments, addition of SLF plus either GM-CSF or IL-3 expanded BFU-E and CFU-GEMM numbers, respectively, by 2.4- to 4.1- and 2.0- to 4.0-fold.

The size of the colonies formed after cells had been in suspension culture for 7 days with SLF with or without a CSF

Table 3. Changes in nucleated cellularity and concentration of MPCs in circulation of 1-day-old infant compared to that infant's cord blood

Parameter(s) evaluated	Myeloid progenitors responsive to	No. of Exp.	Mean ± 1 SEM*	Range
Nucleated cellularity		12	137 ± 12	54–195
CFU-GM colonies	GM-CSF	8	30 ± 11	6–100
CFU-GM colonies + clusters	GM-CSF	8	34 ± 11	7–90
CFU-GM colonies	GM-CSF + SLF	9	34 ± 8	3–88
CFU-GM colonies + clusters	GM-CSF + SLF	9	35 ± 7	4–85
BFU-E	Epo + IL-3	8	46 ± 7	19–80
CFU-GEMM	Epo + SLF	9	41 ± 8	12–74
CFU-GEMM	Epo + SLF + IL-3	9	37 ± 7	7–70

Growth factors were used at the same concentrations as described in the legend to Table 1. Cellularity in millions and MPC numbers in thousands were calculated per ml of blood before comparing values.
*Expressed as % in circulation of 1-day-old infant compared to that infant's cord blood.

Table 4. Comparative expansion of human umbilical cord blood and adult bone marrow MPCs after short-term (7 days) liquid culture with SLF with or without a second cytokine

	Cord blood* (n = 3–5)		Bone marrow* (n = 2)	
	Mean ± 1 SEM	Range	Mean ± 1 SEM	Range
CFU-GM				
Control medium	1.5 ± 0.3	1.0–2.5	1.85 ± 0.35	1.5–2.2
SLF (50 ng/ml)	7.9 ± 1.1	6.5–11.0	8.25 ± 3.65	4.6–11.9
SLF + 2nd cytokine	12.5 ± 1.4	9.0–15.8	10.50 ± 3.90	6.6–14.4
BFU-E				
Control medium	1.2 ± 0.1	0.9–1.8	0.65 ± 0.25	0.4–0.9
SLF	2.2 ± 0.2	1.6–2.5	1.95 ± 0.55	1.4–2.5
SLF + 2nd cytokine	3.6 ± 0.7	2.4–6.9	3.10 ± 1.10	2.0–4.2
CFU-GEMM				
Control medium	1.4 ± 0.3	0.8–2.6	0.5 ± 0	0.5–0.5
SLF	2.7 ± 0.4	1.8–4.3	1.6 ± 0.2	1.4–1.8
SLF + 2nd cytokine	3.5 ± 0.4	2.1–5.1	2.2 ± 0.5	1.7–2.7

The second cytokine added with rhu or rmu SLF (50 ng/ml) in suspension was either rhu IL-3 (100 units/ml), rhu GM-CSF (100 units/ml), rhu G-CSF (100 units/ml), or rhu Epo (1 unit/ml). Low-density (<1.077 g/ml) cells were assessed. The CFU-GM compartment was assessed in agar with rhu GM-CSF (100 units/ml) while the BFU-E and CFU-GEMM compartments were assessed in methylcellulose with rhu Epo (1 unit/ml) and rhu IL-3 (100 units/ml) in all experiments except one cord blood in which all progenitor cells were cultured with rhu Epo + rhu SLF + rhu GM-CSF + rhu IL-3.
*Expressed as increase (-fold) in progenitor cells compared to starting numbers (day 0).

was equal to or greater than the size of colonies formed when fresh cord blood or bone marrow cells were plated directly in semisolid medium.

DISCUSSION

Individual collections of cryopreserved human umbilical cord blood cells have been used successfully in HLA-matched sibling settings as a source of transplantable hematopoietic stem and progenitor cells for engraftment of children with Fanconi anemia and juvenile chronic myelogenous leukemia (5–7). Thus, cord blood will most likely be efficacious in repopulating the bone marrow of children with diverse disorders that are currently treated by bone marrow transplantation. In fact, the development of banks to store frozen cord blood cells is being considered for autologous and allogeneic use (5, 6, 19, 33, 34). Although it is not known how long cells can be maintained in a cryopreserved state, theoretically this should be for at least a normal individual's lifetime. Our present experience has demonstrated that frozen cord blood cells stored for >5 years can be successfully thawed in viable and functional form. However, questions remain regarding the repopulating potential of a single cord blood collection for use in adults.

It is not known how few hematopoietic stem and progenitor cells are needed to repopulate the blood cell system of an adult. When collections of autologous or allogeneic bone marrow cells are made for such purposes, it is usual to collect as many cells as possible (at least 2–4 × 10^8 nucleated cells per kg of recipient body weight). In contrast, while it is possible to enhance somewhat the number of blood cells collected from the umbilical cord at birth by careful attention to the time of clamping the umbilicus and manipulation of the cord and placenta, what is collected is limited by what is present; collection is a one time only possibility.

No assay is available yet that characterizes or quantitates the human marrow hematopoietic repopulating cell, but estimation of the progeny (MPCs) of such a cell in cord blood (4) led to clinical cord blood transplantation in children (5–7). We found that previous numbers of MPCs in cord blood (4, 5, 19) were underestimated by 11- to 15-fold and SLF allowed much greater detection of previously unrecognized cord blood, compared to adult marrow, MPCs. Since SLF is an early-acting cytokine (9–13), this may reflect differences in immature subpopulations of MPCs. Even though aspirations of autologous marrow were maximized to collect as many cells as possible, there was considerable overlap in total MPCs in single cord blood collections compared with the numbers of MPCs in the marrow collections. All or most (13 of 14) of the cord blood collections contained more CFU-GM than that found in the successful autograft collection, which contained the fewest number of CFU-GM of the nine cases of autologous marrow evaluated. With the understanding that numbers of human repopulating cells cannot yet be calculated—but numbers of CFU-GM have been good indicators of bone marrow repopulating capacity (20–25)—these results suggest that single collections of cord blood should have enough such cells to repopulate an adult autologously as well as allogeneically. This requires verification in a clinical cord blood transplant setting. Since this suggestion is based on a relatively limited number of cord blood samples, it would be wise to continue to assess MPC levels in all cord blood collections that are candidates for use in transplantation. This concern is highlighted by a case in which a cord blood we collected for use in an HLA-matched sibling with Fanconi anemia was found to contain a paucity of MPCs. The cells in this collection were subsequently found to manifest the Fanconi genotype and were not used for clinical transplantation (35). It would be of interest to compare numbers of long-term culture initiating cells (36) in cord blood with that in bone marrow. While it is not clear that such long-term culture initiating cells are equivalent to marrow repopulating cells, this assay appears to recognize primitive cells.

The quality of MPCs and earlier cells in cord blood is as important as the quantity of these cells. We have found that SLF enhanced the replating capability of single human cord blood CFU-GEMM colonies (13), a possible indicator of self-renewal capacity. SLF also enhanced the replating capacity of adult human bone marrow CFU-GEMM colonies. However, the replated cord blood colonies gave rise in secondary plates to CFU-GEMM, BFU-E, and CFU-GM colonies, while the replated bone marrow CFU-GEMM colonies gave rise almost exclusively to CFU-GM colonies with only a few BFU-E colonies seen in the secondary replates (13). Thus, it is possible that cord blood may contain greater numbers of an earlier, more immature subset of MPCs than that found in adult bone marrow. This is consistent with our present results, which suggest that most BFU-E detected in cord blood may actually be CFU-GEMM.

We evaluated alternative means to supplement cord blood collections. As one possibility, we determined the content of MPCs in the peripheral blood of children 1 day after birth. While these numbers were still high compared to that present in circulating adult blood, the concentration of MPCs per ml averaged only 30–46% of that found in the cord blood of these infants. Thus, while it may be possible to supplement cord blood collections with the infant's own neonatal blood collected days after birth, this process will probably be of limited usefulness because of the decreased frequency of MPCs in neonatal compared with cord blood and with the small amount of blood that could be safely withdrawn from such young children.

In addition, expansion of cord blood MPCs was investigated in short-term liquid cultures. SLF expanded both cord blood and adult bone marrow MPCs in culture, and this was further enhanced when a second cytokine was added. There was some variability in terms of which second cytokine maximally enhanced SLF expansion of MPCs with individual cord blood samples. However, increasing combinations of cytokines added to semisolid cultures, even at the single cell level, stimulate an increasing number of colonies (37) and, as with mouse bone marrow cells (31), the combination of SLF plus two or more cytokines may allow greater expansion of MPCs than SLF plus one other cytokine. It is likely that MPCs in suspension are being generated from earlier more immature cells, but without an assay for the marrow repopulating cell, it is not yet possible to determine whether numbers of marrow repopulating cells are increasing in the suspension culture. Thus, human MPC expansion studies of others (32) and ourselves are encouraging, but caution is necessary in terms of using such cells for clinical transplantation. Since only a limited number of centers would probably be competent at expansion, an alternative approach might be to expand limited numbers of immature cells of cord blood *in vivo* by administrating growth factors such as SLF and/or CSFs after recipients had received cord blood transplants.

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