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POTENTIAL FOR CLINICAL USE OF VIABLE PLURIPOTENT PROGENITOR CELLS IN BLOOD BANK STORED HUMAN UMBILICAL CORD BLOOD

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Summary

There are indications that a close HLA match may not be necessary when human umbilical cord blood (HUCB) is used to effect a hematopoietic transplant. This was first suggested in 1972 and was further supported by the ability of HUCB to produce mouse survival following lethal irradiation. In China multiple units of HLA unmatched HUCB was utilized successfully in children to effect transplants. A recent publication indicated that newborn rodent blood cells can engraft in adult mice across the H-2 antigens. Furthermore, there recently have been successful transplants with 3 antigens mismatched HUCB. In this study HUCB was stored in polyolefin blood bank bags at 4°C. The storage was similar to that used in routine blood banking. Clonogenic assays were performed at Day 1 and 21 utilizing various growth factors. Replating efficiency was determined on colonies obtained from cord blood that was stored (non-frozen) for 21 days. The functional ability of day 21 old HUCB was determined by its ability to produce survival of lethally irradiated mice. It was found that approximately two-thirds (62.5%) of single primary blast cell colonies in day 21 stored HUCB could generate various types of secondary colonies. In some instances the secondary colonies were counted as high as 42 total mean colonies per single primary colony. These blast cell colonies (CFU-BL) were able to form single and multi lineage colonies composed of virtually every hematopoietic cell types. Animal survival studies were utilized in an effort to determine possible functional ability and were successful in producing fifty-day survival in 54% of lethally irradiated SJL/J mice and 100% ALB/C mice. This study holds the potential of making HUCB available for purposes of marrow transplantation to all who need it. It could further remove most of the moral and ethical issues related to HUCB, reduce to a fraction the cost in providing stem cells for marrow transplantation and potentially allow HUCB to be handled by existing blood banks.

Key Words: **umbilical cord blood, blood bank storage, non-frozen cord blood**

In vitro studies have demonstrated that fresh human umbilical cord blood (HUCB) is rich in pluripotent hematopoietic stem cells. During the first 72 hours after birth, cord blood stored at 4°C has a significant capacity for the formation of hematopoietic stem/progenitor cell colonies [1-7]. There is increasing evidence that human cord blood does not have to be closely matched

for a successful hematopoietic transplant [8,9]. Recently in mice, tissue typing is apparently unnecessary for successful hematopoietic transplantation using cells obtained from the newborn[10]. If cord blood does not need a close HLA match, there is a rather significant increase in its clinical potential. Therefore, we have attempted to determine the viability and functional capacity of human cord blood after 21 days of storage at 4°C which could make storage at -70°C unnecessary except in specific situations. The viability and functional capacity of these human pluripotential stem cells after 21 days of storage could allow for collection, logistics and testing of large quantities of these cells, and thereby make cord blood available for all who might need it for marrow reconstitution. The lack of necessity to have large quantities of cord blood stored frozen would thereby greatly decrease the cost. Furthermore, if cord blood does not need a close HLA match and its pluripotent stem cells retain their viability and functional capacity with blood bank storage at 4°C, this could solve some of the major moral and ethical issues raised by the use of cord blood for marrow transplantation[11,12].

We have studied the in vitro growth pattern of HUCB by a Clonogenic assay, in vivo testing and cell cycle analysis by flow cytometry. Our previous data suggested that the proliferative potential of progenitors in 5-day old stored cord blood detected by flow cytometry was stable or even slightly higher in the presence of the growth factor granulocyte macrophage colony stimulating factor (GM-CSF) or erythropoietin (EPO), although the colony forming capacity decreased significantly[13]. The replating efficiency of stored cord blood was also examined in 7-day old stored cord blood. Primary colony CFU-MIX (Colony Forming Units-MIX) stimulated by the combined cytokines of stem cell factor (SCF) + EPO + GM-CSF achieved the highest replating efficiency of 77%[14]. The presence of CFU-MIX at 7 days suggested that stored HUCB progenitors obtained from 21 day old cord blood also might have the capacity for self-renewal.

Materials and Methods

Thirty umbilical cord blood samples were obtained from the placenta of normal full-term neonates. Collection of cord blood was made into a 50 ml sterile polypropylene test tube containing 5 ml citrate phosphate dextrose (CPD) as an anticoagulant. The volume of cord blood collected varied from 20 to 40cc. Samples were kept at room temperature and sent to the blood bank for storage within 24 hours. Samples of day one (D1) cord blood were examined for Clonogenic assay within 24 hours post delivery. The remaining cord blood was then transferred into a special blood bag made of polyolefin (Cryocyte™ Freezing Container, Baxter Healthcare Corp., Deerfield, IL) and stored in a 4°C blood bank for 3 weeks. These bags allow the transfer of gases[15]. In vitro hematopoietic progenitor colony assays and in vivo survival of day 21 (D21) HUCB transplanted into mice were performed at the end of storage. The temperature and duration of storage was selected because blood banks when using CPD as an anticoagulant store blood for 21 days at 4°C for routine use.

Clonogenic Assay

Stored cord blood was separated by Histopaque 1077 (Sigma, St. Louis, MO) in order to harvest the low density cells. The viability was shown to be 70-80% using trypan blue exclusion. The cells were washed twice with 2% FBS-IMDM (Fetal Bovine Serum-Iscove's Modified Dulbecco's Medium) and plated in a culture system with final concentration of 30% FBS and 0.9% Methylcellulose. Cells were plated with a count of $1x10^5$/ml per culture dish in triplicates. The concentration of growth factors were utilized as follows: human recombinant GM-CSF

100U (Genetics Institute, Cambridge, MA), and human recombinant EPO IU (Amzen, CA), human recombinant IL3 100u (Genetics Institute, Cambridge, MA) and human recombinant stem cell factor 50 ng (Sigma, St. Louis, MO). The growth factors were used individually or combined to stimulate the clonal growth of hematopoietic progenitors. Dishes were incubated for 14 days at 37°C in a fully humidified atmosphere of 8% CO_2.

Colonies were scored under inverted microscopy after two weeks incubation. Cell aggregates of 50 or more were scored as CFU-GM. Cell aggregates that appeared red and contained 50 cells or were composed of at least 3 subcolonies containing a minimum of 10 cells each were scored as a BFU-E. Colonies containing erythroid and non erythroid cells were considered CFU-MIX.

A colony consisting of more than 40 cells that appeared transparent and colorless, with uniform small round shapes, and with no signs of terminal differentiation, was considered a blast colony (CFU-BL)[16-18].

Replating Efficiency

The colonies were counted in a laminar flow hood in order to maintain sterility. After completing the colony counting, a single isolated colony was selected under inverted microscopy and lifted by using a 200 ul micropipet with the volume adjusted to 30 ul. All the colonies replated were taken from the dishes that were stimulated with growth factor GM-CSF plus EPO plus IL-3. The single primary colony was then suspended into 0.64ml of 56% FBS-IMDM and gently mixed for dispersion of the colony cells. The replating system consisted of the same concentration as the primary culture with a final addition of 0.56 ml of 1% methylcellulose. Various growth factors GM-CSF, EPO, IL3 and SCF, alone or combined were added into the plates. After 2 weeks of culture at 37°C, 8% CO_2 incubator, secondary colonies were scored.

Transplantation

Mice receiving human cord blood were given rabbit anti-Asialo GM_1 antibody (Wako Chemicals, Richmond, VA). Injections of 100 ul of this antibody intravenously depletes NK effector cells [19,20]. All mice that received cord blood infusion were given anti-Asialo GM_1 antibody 24 hours before irradiation.

Irradiation was delivered from 8 MeV X-ray beam of a linear accelerator (Philips SL 75-20) using a 25 x 25 cm field with a dose rate of about 400 cGy/minute. Animals were restrained during irradiation in a Lucite box measuring 19.8 x 15.9 x 6.5 cm, which holds 10 mice in individual compartments. Polystyrene blocks of 5.22 and 5.46 cm thickness were placed above and below the box respectively to provide dose build-up and back scatter. The source surface distance of the irradiation assembly was 89 cm with mice at a mean distance of 100 cm from the X-ray source.

All irradiated animals were housed in a laminar air flow compartment with sterilized cages, food, water, and bedding for the duration of the experiment and given antibiotics (sulfamethoxazole and trimethoprim) in their drinking water.

Two different strains of mice were used to investigate survival post lethal irradiation.

SJL/J mice (6-10 weeks old) were obtained from the Jackson Laboratory (Bar Harbor, Maine), and were housed in the AAALAC - Accredited Research Animal Facility of New Jersey Medical School. Except for a diminished number of NK cells, the SJL/J mice are immunocompetent. The SJL/J mice received a lethal dose of 950 cGY of irradiation. The first transfusion of hematopoietic cells was carried out within two hours following irradiation. Syngeneic bone marrow cells were obtained by direct repeated flushing of the bone marrow cavity from the tibias of normal SJL/J donor mice using 2 ml of normal saline delivered from a syringe through a 27 g needle. Each mouse received 4 x 10^6 mouse bone marrow nucleated cells. SJL/J mice transplanted with D21 human cord blood were divided into two groups: 24 mice received low density cells, and 20 mice received buffy coat nucleated cells (BC). The low density cells were isolated by Ficoll Hypaque 1077. Cord blood buffy coat nucleated cells were harvested by centrifugation at 1000 rpm for 20 minutes. These cells were washed 3 times with sterilized PBS and then suspended in normal saline depending on the cell concentration for injection. Injection was intravenously through the inner cantus of the eye. The range of transplanted cord blood cells for the SJL/J mice varied. The first group received 10-15.3 x 10^6 low density cells and the second group received 11.2-14.4 x 10^6 buffy coat nucleated cells per each mouse.

BALB/C mice were used as an alternate strain for the study of survival following lethal irridation of 900cGY. Within 2 hours post irradiation, five BALB/C mice received 9.6 x 10^6 HUCB low density cells. There were five controls with radiation only.

Morphological Findings

Morphological findings were evaluated on the D21 stored cord blood taken for Clonogenic assays from the polyolefin blood bags. One half microliter of unseparated whole cord blood was examined for the leukocyte count and differentiation. The cord blood smears were stained by May Grunwald-Giemsa stain.

Results

The results of clonal growth of thirty HUCB samples are shown in Table 1. The multi-potent progenitors of D1 cord blood responding to cytokine GM-CSF developed into committed granulocyte-macrophage colonies. With the addition of cytokine IL-3 to the D1 culture dish containing GM-CSF, the colony count did not increase but the size of CFU-GM was slightly enhanced. However, no BFU-E or CFU-MIX colonies developed. Comparatively, when stimulated by EPO cytokine alone, a large number of BFU-E, and also many CFU-GM and CFU-MIX colonies were found. This unique feature of in vitro growth of fresh cord blood suggested an endogenous production of hematopoietic growth factors within the culture dish. The combination of IL-3 and EPO did not show additional colony formation in the D1 culture. The incidence of total erythroid colonies including BFU-E + CFU-MIX was 79 in the presence of EPO alone, versus 78 with the combined use of EPO and IL-3. An overlap of the progenitor population[21-23] responding to the individual cytokine EPO and IL-3 may explain this finding.

Compared to fresh cord blood, the colony growth pattern of D21 progenitor cells decreased significantly. The mean CFU-BL plus CFU-GM colonies was only 0.11 in D21 cord blood

versus 68 CFU-GM colonies when stimulated by cytokine GM-CSF alone. The mean total colonies including GM-CFU, BFU-E and CFU-MIX was 20.6 in D21 cord blood versus 103.6 in D1 cord blood in response to combined growth factors IL-3 plus EPO. The morphological appearance of the cells in D21 were atypical and comparative counts could not be made accurately between D1 and D21. Differential counts could not be done by the coulter counter but required direct visualization of the smears due to deterioration of cells. The majority of nucleated cells in D21 cord blood were small mononuclear cells, similar to lymphocytes with a mean percentage of 86% with a range of 76-94%. Only 2-8% band shaped neutrophils were visualized. Poikilocytosis and nucleated red cells were common.

Table I: Primary Colony Growth of Progenitor Cells in 21-Day Stored HUCB*
Colonies per 10^5 LDC, Mean ± SE

In Vitro Treatment	D1				D21			
	CFU-BL	CFU-GM	BFU-E	CFU-MIX	CFU-BL	CFU-GM	BFU-E	CFU-MIX
GM-CSF	0**	68±3.9	0	0	0.11±.06	0	0	0
GM-CSF +IL3	0**	66±2.6	0	0	0.61±3.9	3.18±.96	0	0
EPO	0**	24±2.5	36±4.0	43±2.4	0	0.21±.09	0.86±.19	2.38±.50
EPO+IL3	0**	25.6±1.9	56±3.9	22±1.7	0.41±.23	2.36±.44	4.30±.78	13.61±1.90
GM-CSF +EPO +IL3	0**	ND	ND	ND	0.98±.35	2.50±.63	6.09±1.44	13.04±1.82

LDC - low density cells
ND-NOT DONE
CYTOKINES: GM-CSF 100U, EPO IU, IL-3 100U
*Human cord blood obtained from 30 individual babies were cultured both fresh and following 21 days storage. Culture were grown in the presence of the indicated factors. In the case of those cultures containing GM-CSF and EPO an additional 43 cord blood samples were analyzed.
** Exact number of colonies may be obscured and were not definitely identified.

There was no eventual difference in colony size between D1 and D21. Giant colonies could be found in D21 cord blood but not as many as in day 1 cord blood. In D21 cord blood a statistical difference was found ($P < 0.0005$) in the total erythroid colonies including BFU-E and CFU-MIX when growth factor IL3 was added to EPO as compared to EPO alone. In D1 cultures, however, the total colony forming of BFU-E and CFU-MIX stimulated by EPO or EPO plus IL3 was similar. As the result of in vitro cultures of D21 cord blood, the addition of cytokine IL3 combined to EPO or GM-CSF alone produced additive colony growth as measured by both colony number and size when compared to EPO or GM-CSF alone.

A total of 450 single primary colonies were lifted and replated individually from 21 samples of 3-week stored human umbilical cord blood. Secondary colonies were formed in 138 replated dishes. The results of replating experiments are presented in Table 2. In general secondary

colonies appeared smaller in size and consisted of smaller cells. The incidence of secondary colony formation depended on the characteristics of the primary replated colony and the cytokines on which the secondary colonies were grown. Replating efficiency was defined as the percentage of primary replated cells which gave rise to at least 1 secondary colony. Eighty-eight primary CFU-BL colonies with the stimulation of combined cytokines GM-CSF+ EPO+ IL3+ SCF achieved the highest replating efficiency of 62.5% and produced the largest numbers of 44 secondary colonies per single primary replated colony. Among those the majority of secondary colonies appeared to be CFU-MIX; however, a limited number of differentiated progenitors BFU-E and CFU-GM were also formed (Table 2). Eleven out of 88 primary CFU-BL (data not shown) colonies had extensive potential capacity with more than one hundred secondary colonies forming per each replated CFU-BL. Some huge secondary colonies containing >1000 cells were found although most of them were smaller than the primary colony. The replating efficiency of primary CFU-BL generating into secondary CFU-BL was increased with the addition of cytokine SCF in comparison to the capacity of CFU-BL lacking the combined stimulating effect of SCF. Without SCF the replating efficiency was reduced to 16.7% and the mean value of total secondary colony formation was only 8.75 per primary replated colony.

The frequency of 212 CFU-MIX primary colonies replated with the addition of the combined cytokines GM-CSF +EPO + IL3 + SCF was lower compared to the CFU-BL primary colonies, with a replating efficiency of 33.5% and a dramatic decrease in number of total secondary colony formation (Table 2). Various patterns of secondary colonies were found and CFU-MIX was the common pattern of secondary progenitors. The secondary colonies were poorly formed and appeared small to minute in size when lacking the stimulation of stem cell factor. No secondary colonies were seen when the committed progenitors CFU-GM or BFU-E were replated even in the presence of cytokine SCF factor in combination with the other growth factors.

A total of 72 SJL/J mice received 950 cGY of irradiation (Fig. 1). Out of 15 radiation control mice, two (13%) survived to 7 weeks post-irradiation. The 7 week survival of 7 out of 8 syngeneic bone marrow transplant mice was 87.5%. Four out of 5 mice receiving fresh HUCB transplant at a dose of 15 x 10^6 nc per mouse survived (80%) at 7 weeks. Twenty four mice received 21-day stored HUCB transplant at the dose of 10-15.3 x 10^6 low density cells per mouse over a 4 day period. Fourteen mice (58%) were alive 3 weeks post transplant. At 7 weeks the survival was 54% (Fig. 1). Twenty mice received 21-day stored HUCB transplant at the dose of 11.2-14.4 x 10^6 buffy coat nucleated cells per mouse over a 4-day period. One half of the mice survived 3 weeks post-irradiation. The 7 week survival was 40% (Fig. 1).

Ten mice of BALB/C strain were irradiated with 900cGy and five were transplanted (Fig. 2). Four out of five radiation control mice died within two weeks while all five mice infused with low density 21 day old human cord blood cells ranging from 6.9 - 9.6 x 10^6 survived (Fig. 2). At 10 weeks, survival of transplanted mice was 100%.

Table 2: Replating Efficiency of 21-Day Stored Human Cord Blood
Mean = ± SE

PRIMARY COLONIES			*SECONDARY COLONIES*					
1° Colony Replated	n	In Vitro Treatment	% Replates with at least 1 Secondary Colony	# Secondary Colonies per Replated Colony				Total # of 2° Colonies per 1° Replated Colony
				BL	GM	E	MIX	
CFU-MIX	212	GM - CSF + EPO +IL3 + SCF	33.5	0.49	1.39	0.84	8.20	10.92 ± 2.58
CFU-MIX	108	GM - CSF+ EPO+ IL3	12	0	0.46	0.16	0.68	1.30 ± 0.70
CFU-BL	88	GM - CSF + EPO+IL3 + SCF	62.5	6.6	1.8	3.74	31.74	43.88 ± 9.88
CFU-BL	12	GM - CSF + EPO + IL3	16.7	0	8.75	0	0	8.75 ± 5.07
CFU-GM	14	GM - CSF + EPO + IL3 + SCF	0	0	0	0	0	0
CFU-GM	2	GM - CSF + EPO + IL3	0	0	0	0	0	0
BFU-E	11	GM - CSF + EPO + IL3 +SCF	0	0	0	0	0	0
BFU-E	3	GM - CSF + EPO + IL3	0	0	0	0	0	0

Cytokines for secondary colonies: GM-CSF 100U, EPO 1U, IL3 100U, SCF 50 ng

Discussion

Pluripotent hematopoietic stem cells are defined functionally by their ability of self renewal and to give rise to multilineage blood cells[24]. Under steady state conditions the majority of stem cells are quiescent and only a small number of stem cells enter into cell cycle with subsequent expansion and differentiation[25]. There is not yet a direct assay for detecting human pluripotential hematopoietic stem cells. In vitro clonogenic assays are commonly used to reflect the stem cell populations. Fresh HUCB is found to possess as many hematopoietic progenitors as normal adult bone marrow [3,26] and with a higher proportion of primitive progenitors [14]. Our laboratory findings have been similar to or higher than the published data[14,5] on this subject.

The unique pattern of in vitro growth of Day 1 cord blood with EPO alone suggested an endogenous stimulation of growth factor derived from the progenitors in fresh cord blood. Within the culture dishes endogenous GM-CSF and IL-3 production has been identified[27]. It appears that EPO must play an essential growth promoting role to trigger the progenitor cell expansion and differentiation in fresh HUCB. In our studies the combination of EPO plus IL-3 failed to stimulate the formation of additional colonies in cultures of D1 cord blood; however, IL-3 is a required co-factor to obtain maximal development of colonies at day 21.

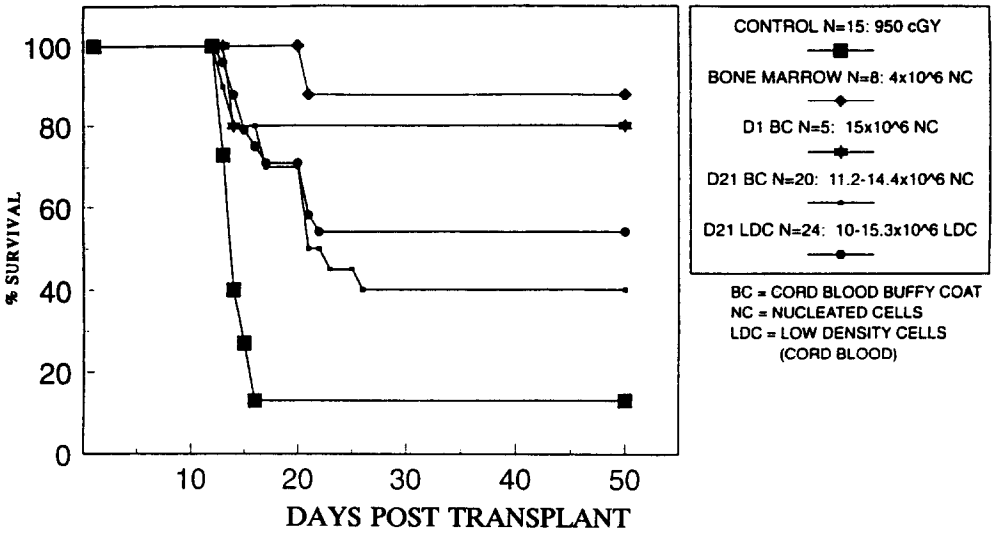

FIG. 1

SJL/J MICE SURVIVAL FOLLOWING LETHAL IRRADIATION USING 21-DAY STORED HUCB

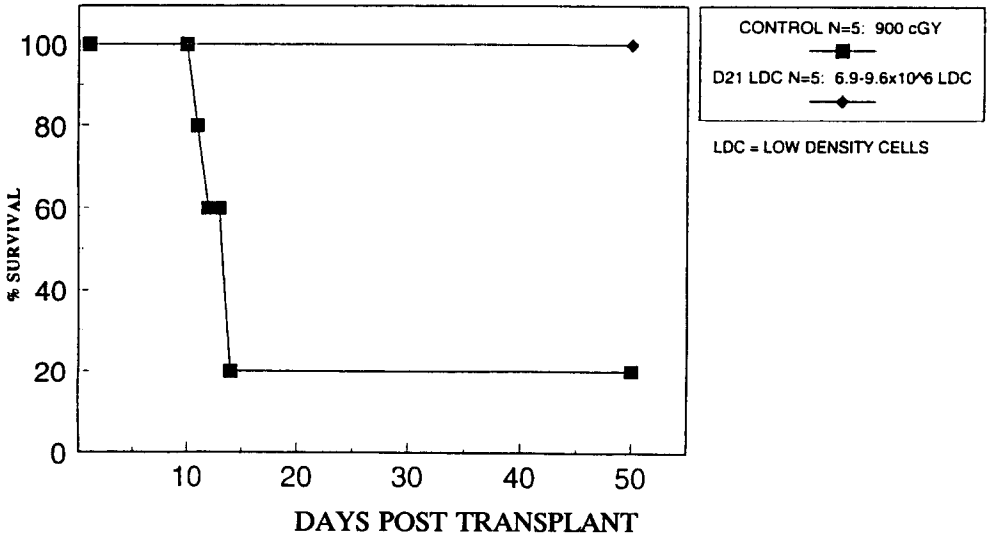

FIG. 2

BALB/C MICE SURVIVAL FOLLOWING LETHAL IRRADIATION USING 21-DAY STORED HUCB

It is generally accepted that the stem cell compartment is heterogeneous and hierarchical[14,28]. There are at least two subpopulations of stem cells differing in early short-term repopulating ability versus later long-term hematopoietic reconstitution. Only a few pluripotent stem cells participate in the repopulation and long-term maintenance of engrafted hematopoietic and lymphoid tissue. The stem cell subpopulation responsible for the long-term engraftment are early primitive cells low in number[24] and may have a long-term survival. These stem cells may represent the most primitive of the pluripotential stem cells and have very few if any recognition antigens. We have given the name of this cell the "Barashis cell" from the first word in the Bible[12] meaning "in the beginning."

In preliminary studies, to further identify these primitive pluripotent stem cells, ten samples of day 21 cord blood were analyzed for both immunophenotyping and clonogenic assay. No direct relationship was found between CD 34^+, the characteristic markers of hematopoietic progenitors in whole cord blood and the percentage of colony formation in the clonogenic assays (data not shown). The hematopoietic stem cells however in D21 stored cord blood may be the long-term survival sub-population, at least when compared to D1 hematopoietic cells. The colony formation achieved in D21 cord blood was significantly less than in D1 samples as shown in Table 1. In D21 cord blood progenitors committed to differentiate into granulocyte macrophage colonies decreased significantly to near zero after exposure to cytokine GM-CSF alone. Similarly, only a few colonies were seen in the culture dishes containing the growth factor EPO alone. But a statistically significant difference between the colony formation of EPO versus EPO plus IL3 ($P<0.0005$), and GM versus GM plus IL-3 ($P<0.001$) was found (Table 1). This indicated that D21 hematopoietic progenitors were IL-3 dependent. Since IL-3 acts in the early development of progenitors[24,29], the IL-3 dependent D 21 progenitors might be in a more primitive stage than D1 progenitors.

Replating of single colonies was utilized as a measure of self-renewal capacity of hematopoietic stem cells[16]. About two-thirds (62.5%) of single primary blast cell colonies in D21 stored cord blood could generate various kinds of secondary colonies. The secondary colonies were counted as high as 42 total mean colonies per single primary colony. The CFU-BL derived colonies were able to form single and multilineage colonies composed of virtually every hematopoietic cellular type (Table 2). However, without the combination of SCF only a small amount of secondary GFU-GM colonies could be found. This finding may reflect the existence of limited self-renewal but lack of differentiation without the stimulation of SCF. Stem cell factor[30] appears to be vital for maintaining extensive self renewal capacity, and essential for the differentiation of the blast cell colonies.

Human blast cell colonies are reported in the literature to be detected on day 21-28 of the cultures of fresh cord blood and bone marrow and have a 40 to 75% yield of secondary colonies on replating[17]. Blast colonies may be confused with other immature colonies of other lineage before 14 days[18] of culture. The studies reported herein were on cultures incubated for only 14 days. This may account for the lack of identifiable blast cell colonies of cultures made from D1 cord blood (Table 1).

The small number of blast cell colonies recovered on day 21 specimens are not unexpected as we suspect that only the most primitive stem cells have survived [Barashis Cell (12)] while the committed progenitor cells have gone on to differentiate and mature.

On a comparative basis one published article concerning both fresh and previously frozen cord blood had a replating efficiency of 27 to 46% with 10.2± 31 CFU GM colonies and 18.0± 7.1 CFU GEM per replated colony[31]. In our studies on 21 day old blood bank stored HUCB using combined cytokines GM- CSF, EPO, IL3 and SCF, the replating percentage of replated CFU-BL colonies was 62.5 with an average of 1.8 CFU-GM colonies and 31.74 CFU-MIX colonies per replated colony.

An additional in vivo assay for 21D cord blood stem cell function was to employ these cells as a factor rescuing mice from lethal irradiation. The promising survival of xenogeneic transplant from human to mice may also imply the low immunologic component[32] in 21 day stored cord blood. We believe that following lethal irradiation, the human cord blood serves only as a temporary bridge allowing the animals' marrow to reconstitute itself but a small number of human cord blood cells or human DNA survives indefinitely in the animals' tissues[33].

With evidence that hematopoietic stem cells in 21 day old HUCB are both viable and functional, with both old[12] and recent publications indicating human umbilical cord blood does not have to be closely matched, clinically[8,9] and experimentally[10]; the possibility of human cord blood becoming readily available for all who need it may become a reality.

In conclusion, our preliminary findings demonstrated that hematopoietic stem cells in cord blood are heterogeneous. There are a few hematopoietic pluripotent progenitors in cord blood that survived up to 21 days at 4°C. These cells appeared morphologically like small lymphocytes. The in vitro clonal assay response to cytokines of D21 hematopoietic stem cells was different from D1 hematopoietic stem cells. D21 cord blood contained early primitive pluripotent progenitors with extensive renewal capacity; clonal assay showed they were IL-3 dependent and SCF essential for their subsequent development. We suggest that there is a subpopulation of stem cells in cord blood with long-term survival, low in distribution, cytokine IL-3 dependent and SCF essential. This subpopulation is probably responsible for cord blood's ability to repopulate hematopoietic tissue post ablation of bone marrow.

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