STUDY OF HEMATOPOIETIC PROGENITOR CELLS, HEMATOLOGICAL VALUES AND LYMPHOCYTE SUBSETS IN CORD BLOOD: APPLICATION FOR CORD BLOOD TRANSPLANTATION

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Abstract. Hematological values, lymphocyte subsets and hematopoietic progenitor cells from normal term cord blood samples were studied, compared with normal adult blood, and analysed to determine whether a single collection of cord blood is sufficient for transplantation in adults. The parameters were assayed by automatic cells counter, flow cytometry and semisolid cell culture. All of the hematological values except RBC and MCHC were higher than in normal adult blood. Sex had an influence on RBC, Hb, Hct, Plt and reticulocyte counts. For lymphocyte subsets, all of the absolute $CD3^+$, $CD4^+$, $CD8^+$ counts and T helper: suppressor ratio were higher than those of adult blood. All of the hematopoietic progenitor cells in cord blood were also higher than in adult blood. The mean volume of cord blood for each collection was 80.75±4.81 ml and the mean numbers of nucleated cells, CFU-GM and $CD34^+$ were $13.51\pm0.38\times10^8$ cells, $4.33\pm0.66\times10^5$ colonies and $42.65\pm7.00\times10^6$ cells respectively. This 80 ml of cord blood would contain sufficient marrow repopulating cells for a recipient weighing about 20 kg. Recently developed technology, including *ex vivo* expansion may even permit transplants in adults.

INTRODUCTION

It has been shown by Broxmeyer *et al*, (1990) and others (Broxmeyer *et al*, 1989; Abboud *et al*, 1992; Nakahata *et al*, 1982) that both term and preterm umbilical cord blood contain a significantly higher number of early and committed progenitor cells when compared with adult peripheral blood. This fact has enabled cord blood to be used successfully for hematopoietic reconstitution in children with various hematological disorders (Gluckman *et al*, 1989; Vilmer *et al*, 1992; Wagner *et al*, 1993). Umbilical cord blood posses a number of advantages over bone marrow and peripheral blood as a source of allogeneic progenitor cells such as wide availability, ease and low cost of procurement, unbiased ethnic participation, low incidence of graft versus host disease (GVHD) and a decreased risk of transmission of infection.

The purpose of this study is to compare the hematological values, lymphocyte subsets and hematopoietic progenitor cells in cord blood with normal adult peripheral blood and analyse whether a single collection of cord blood is sufficient for transplantation in an adult.

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MATERIAL AND METHODS

Subjects

Normal and term cord blood samples were obtained from delivery unit, Department of Obstetric and Gynecology, Ramathibodi Hospital, normal adult blood samples were collected from healthy volunteer and the donors from Blood Bank. Normal subjects were screened by study history, blood smear and red cell indices.

Collection of umbilical cord blood

Immediately upon delivery of the infants, the umbilical cord was doubly clamped and transected. The infant was removed and blood was collected from the maternal end of the transected cord while the placenta remained *in situ*. In some cases blood was obtained from the removed placenta by needle aspiration of vessels on the fetal surface.

Analysis of hematological values

Blood were collected in EDTA tubes and hematological indicies were determined by Coulter Counter model JT3. Blood films were made and stained with Wrights' stain, 100 cell differential counts were done.

Analysis of hematopoietic progenitor cells *in vitro*

Blood samples were diluted with an equal volume of MEM α medium. Light density cells were separated by centrifugation over Ficoll-Hypaque, $5x10^5$ cells were used in each assay.

CFU-GM assay was performed in 1.65% agar culture medium (agar Noble) that included MEM α medium with 20% (vol/vol) heat-inactivated human AB serum in the presence of 10% CM 5637 as growth factors.

BFUE and CFU-GEMM assay was performed in MEM α medium containing 0.9% methyl cellulose and supplemented with 30% normal human AB serum, 10% CM 5637, 1% deionized bovine serum albumin, $5x10^{-5}$ M 2-mercaptoethanol, 1.5 mM L-glutamine and 1 unit recombinant human erythropoietin.

The cultures were incubated at 37°C in an atmosphere of 5% CO_2 in air with extra humidity. Colonies (>40 cells per aggregate) and clusters (10-40 cells per aggregate) were scored after 10 days of incubation for CFU-GM and 14 days for BFUE and CFU-GEMM. Results were expressed as colonies plus clusters. The colonies were scored with the aid of an inverted microscope. Each experiment was carried out in duplicate in 35 mm tissue culture dishes.

Flow cytometric analysis

$CD34^+$, $CD3^+$, $CD4^+$, and $CD8^+$ counts were performed by direct immunofluorescence staining using flow cytometric technique on a Facsort flow cytometer (Becton Dickinson, USA). This technique was carried out by using 50 µl of cell suspension containing $1x10^6$ WBC, labelled with monoclonal antibody fluorescein dye as followed, 30 minutes at 2-8°C for $CD34^+$, 15 minutes at room temperature for $CD3^+$, $CD4^+$ and $CD8^+$ with 20 µl of CD34 (Anti HPCA-2) PE/CD45 FITC, CD3 FITC/CD4 PE/CD45 Per CP and CD3 FITC/CD8 PE/CD45 Per CP respectively. Mouse IgG_1 was used as control for background non specific staining.

After labeling, red blood cells were lysed by adding 1 ml of FACS lysing solution for 10 minutes at room temperature in the dark. The suspensions were washed with PBS and fixed with 0.5 ml of 1% paraformaldehyde, mixed throughly and analysed.

Statistical analysis

Unpaired T-test and Mann-whitney U test were used to compare means.

RESULTS

Hematological values in cord blood samples

The mean±standard diviation of hematological values are shown in Table 1. All parameters except RBC and MCHC in cord blood samples were significantly higher than in normal adult blood. Sex was found to have effects on RBC, Hb, Hct, Plt and reticulocyte but not the other parameters (Table 2).

Numbers of hematopoietic progenitor cells

Various hematopoietic progenitor cells from cord blood were significantly higher than normal adult blood as shown in Table 3.

Comparison of immunophenotyping of cord blood and adult values

Table 4 shows mean percentages of the lymphocyte subpopulation in both cord and adult blood samples. Significant differences were identified in the helper: suppressor ratio and in the two lymphocytes subsets analysed. The percentage of T-helper ($CD4^+$) was significantly increased in cord blood compared to adult blood whereas the percentage of cytotoxic T cells ($CD8^+$) was significantly decreased. Absolute counts for T cells and their subsets were higher in cord blood than those found in adults.

Cellularity and number of hematopoietic progenitor cells in human umbilical cord blood

Twenty-four cord blood samples were assessed for volume of blood collected, number of unfrac-

Table 1
Comparison of hematological values in 99 cord blood and 66 normal adult blood samples.

Parameter	Cord blood	Adult blood
WBC 10^9/l	16.01 ±0.43*	7.29 ±0.21
RBC 10^{12}/l	4.57 ±0.05*	4.90 ±0.08
Hb g/dl	15.50 ±0.14*	13.87 ±0.21
Hct%	47.22 ±0.43*	42.59 ±0.59
Plt 10^9/l	291.38 ±7.01*	260.64 ±6.31
MCV fl	103.73 ±0.64*	87.22 ±0.81
MCH pg	34.06 ±0.24*	28.41 ±0.31
MCHC g/dl	32.83 ±0.12	32.52 ±0.14
Neutrophil 10^9/l	8.26 ±0.25*	4.15 ±0.16
Lymphocyte 10^9/l	5.18 ±0.17*	2.40 ±0.09
Monocyte 10^9/l	1.35 ±0.07*	0.37 ±0.03
Reticulocyte%	5.13 ±0.12*	1.70 ±0.08
NRC/100 WBC	4.29 ±0.43*	0.00 ±0.00

Mean±1 SEM: Difference from adult blood with $p<0.001$*

Table 2
Hematological values by sex between cord blood and adult blood.

Parameter	Cord blood		Adult blood	
	Male (n=50)	Female (n=46)	Male (n=35)	Female (n=31)
RBC 10^{12}/l	4.67±0.06[a,b]	4.44±0.08	5.34±0.09[d]	4.41±0.06
Hb g/dl	15.92±0.19[a,b]	15.00±0.21[c]	15.07±0.18[d]	12.51±0.21
Hct%	48.20±0.58[a,b]	46.01±0.62[c]	46.05±0.51[d]	38.67±0.57
Plt 10^9/l	269.74±7.37[a,b]	313.76±11.70[c]	252.31±8.93	270.03±8.71
Reticulocyte%	4.93±0.15[a,b]	5.42±0.18[c]	1.67±0.11	1.72±0.12

Mean±1 SEM: [a]Difference from female cord blood with $p<0.05$; [b]Difference from male adult blood with $p<0.01$; [c]Difference from female adult blood with $p<0.01$; [d]Difference from female adult blood with $p<0.001$

Table 3
Number of hematopoietic progenitor cells in cord blood and normal adult blood.

	CFU-GM	BFUE	CFU- GEMM	CFU-GM	BFUE	CFU- GEMM	CD 34+	
Group	colonies/5x10^5 MNC (n)			colonies/μl (n)			% (n)	cells/μl (n)
Cord blood	410.11±56.55[a] (47)	1504.86±147.26[a] (50)	96.24±14.41[a] (50)	5.36±0.82[a] (47)	20.21±3.01[a] (50)	1.30±0.25[a] (50)	0.30±0.03[a] (41)	52.82±8.67[a] (41)
Adult blood	21.79±3.51 (47)	139.54±20.52 (37)	3.22±0.91 (37)	0.13±0.03 (47)	0.81±0.14 (37)	0.02±0.01 (37)	0.02±0.00 (64)	1.13±0.13 (64)

Mean±1 SEM: Difference from adult blood with $p<0.001$[a]

Table 4
Percentage and absolute lymphocyte subsets from 89 cord blood and 64 normal adult blood samples.

Group	lymphocyte	CD3+	CD4+	CD8+	CD4+ / CD8+	CD3+	CD4+	CD8+
	%					10^9/l		
Cord blood	33.05±0.90	66.40±1.11	47.39±0.98[a]	19.38±0.66[a]	2.78±0.14[a]	3.41±0.12[a]	2.43±0.09[a]	0.99±0.05[a]
Adult blood	33.71±1.17	66.94±0.86	38.63±0.79	26.95±0.72	1.51±0.06	1.62±0.06	0.93±0.04	0.65±0.03

Mean±1 SEM: Difference from adult blood with $p<0.001$[a]

Table 5
Cellularity and number of hematopoietic progenitor cells in 24 human cord blood samples (mean ± 1 SEM).

Volume (ml)	Nucleated cells $x10^8$	Hemopoietic progenitor cells $x10^5$			
		CFU - GM	BFUE	CFU - GEMM	CD 34^+
80.75±4.81	13.51±0.38	4.33±0.66	16.32±2.43	1.05±0.21	42.65±7.00

tionated nucleated cells and absolute number of hematopoietic progenitor cells as shown in Table 5.

DISCUSSION

The study has shown hematologic differences between cord and adult blood. The rise in hemoglobin and hematocrit in cord blood may be due to the movement of plasma from the intravascular to the extravascular space. Sex has an influence on RBC, Hb, Hct, and Plt in both cord and adult blood. For the WBC, the early studies (Shimoda *et al*, 1992; Russell *et al*, 1993; Laver *et al*, 1990) have demonstrated high levels of G-CSF and GM-CSF in cord blood and the early neonatal period, and the placenta is a rich source of colony stimulating activity (Yuen *et al*, 1986; Saito *et al*, 1993). These growth factors may stimulate the production of WBC, neutrophil and monocyte in neonates at birth, the changes in blood volumes and hemodynamics before and after delivery may induce increased levels of serum G-CSF by the placenta, leukocytosis as well (Ishii *et al*, 1995). So the high proportion of WBC and stem cells in cord blood in our study was confirmed. Broxmeyer *et al* (1990) and others have demonstrated that CFU-GM and CFU-GEMM were significantly increased in term umbilical cord blood compared to adult peripheral blood. Lim *et al* (1994) analysed the influence of delivery and found that stress during delivery may induced leukocytosis, $CD34^+$ cells as well as hematopoietic progenitor cells but reduced percentage of T cells in umbilical cord blood. Benedetti (1996) described that under physiologic conditions, $CD34^+$ cells comprise 0.1-0.5% and 0.001-0.01% of nucleated cells in cord blood and peripheral blood respectively, these are consistant with our data, although several workers presented that approximatly 1% of nucleated cells present in unfractionated cord blood express $CD34^+$ cells (Isoyama *et al*, 1996; Sutherland *et al*, 1994).

Among the $CD34^+$ subsets, $CD34^+38^-$ and $CD34^+DR^+$, which have high proliferative potential and enriched with primitive hematopoietic progenitors, were significantly higher than has been reported for adult marrow (Payne *et al*, 1995) and mobilized peripheral blood stem cells (Ho *et al*, 1996).

The absolute lymphocyte count was greater in cord blood than in adult blood, but the percentages of lymphocyte and T cells ($CD3^+$) in cord blood in our study were not different from normal adult blood. $CD4^+$ cells were higher and $CD8^+$ cells were lower, resulting in an increased in $CD4^+$: $CD8^+$ ratio, this agreed with Apperley (1994) and Keever (1993). Other studies (Beck *et al*, 1994; Motley *et al*, 1996; Cairo *et al*, 1997; Harris *et al*, 1992) however, reported a lower percentage of T cells than normal adult blood. Harris *et al* (1992) found that cord blood T cells had an increased number of unprimed or naive T cells ($CD45RA^+$) and a decreased population of mature or primed T cells ($CD45RO^+$) compared with that of adult peripheral blood T cells. In addition, their functionality were immature as shown by minimal response to stimulation with IL-2, PHA or alloantigens. These was confirmed by the others (Beck *et al*, 1994; Motley *et al*, 1996; Cairo *et al*, 1997). Thus cord blood cells may not be as capable of mediating GVHD after transplantation.

According to Cairo *et al* (1997) and Denning *et al* (1996) the medium numbers of nucleated cells, CFU-GM and $CD34^+$, in the graft on the basis of patient's body weight were $4.7x10^7$/kg, $1.7x10^4$/kg and $2x10^5$/kg respectively. From our study, the collection of 80.75±4.81 ml of cord blood contained $13.51±0.38x10^8$ nucleated cells, $4.33±0.66x10^5$ CFU-GM and $42.65±7.00x10^5$ $CD34^+$ cells. Thus, 80 ml of cord blood would contain sufficient number marrow repopulating cells for recipient weighs about 20 kg. Recently developed technology, including *ex vivo* expansion may even permit transplants in adults.

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REFERENCES

Abboud M, Xu F, Lavia M, Laver J. Study of early hematopoietic precursors in human cord blood. *Exp Hematol* 1992; 20: 1043-7.

Apperley JF. Umbilical cord blood progenitor cell transplantation. *Bone Marrow Transplant* 1994; 14: 187-96.

Beck R, Lam PO, Tang PR. Comparison of cord blood and adult blood lymphocyte normal ranges: A possible explanation for decreased severity of graft versus host disease after cord blood transplantation. *Immunol Cul Biol* 1994; 72: 440-4.

Benedetti F. CD 34+ cells: Biological aspects. *Tumori* 1996; 82 (2 suppl 2): S3-13.

Broxmeyer HE, Douglas GW, Hangoc G, *et al.* Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci USA* 1989; 86: 3828-32.

Broxmeyer HE, Gluckman E, Auerbach A, *et al.* Human umbilical cord blood: A clinical useful source of transplantable hematopoietic stem/progenitor cells. *Int J Cell Cloning* 1990; 8: 76-91.

Cairo MS, Wagner JE. Placental and/or umbilical cord blood: An alternative source of hematopoietic stem cells for transplantation. *Blood* 1997; 90: 4665-78.

Denning KP, Donaldson C, Nicol A, Bradley B, Hows J. Optimal processing of human umbilical cord blood for clinical banking. *Exp Hematol* 1996; 24: 1394-401.

Gluckman E, Broxmeyer HE, Auerbach A, *et al.* Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA identical sibling. *N Engl J Med* 1989; 321: 1174-8.

Harris DT, Schumacher MJ, Locascio J, *et al.* Phenotypic and functional immaturity of human umbilical cord blood T lymphocytes. *Proc Natl Acad Sci USA* 1992; 89: 10006-10.

Ho AD, Young D, Maruyama M, Corringham RE, *et al.* Pluripotent and lineage committed CD34+ subsets in leukapheresis products mobilized by G-CSF, GM-CSF VS a combination of both. *Exp Hematol* 1996; 24: 1460-8.

Ishii E, Masuyama T, Yamaguchi H, *et al.* Production and expression of granulocyte and macrophage colony stimulating factors in newborns: Their roles in leukocytosis at birth. *Acta Haematol* 1995; 94: 23-31.

Isoyama K, Yamada K, Hirota Y, Ishikawa K, Imai M, Notake Y. Study of the collection and separation of umbilical cord blood for use in hematopoietic progenitor cell transplantation. *Int J Hematol* 1996; 63: 95-102.

Keever CA. Characterization of cord blood lymphocyte subpopulations. *J Hematother* 1993; 2: 203-6.

Laver J, Duncan E, Abboud M, *et al.* High levels of granulocyte and granulocyte macrophage colony stimulating factors in cord blood of normal full-term neonates. *J Pediatr* 1990; 116: 627-32.

Lim FT, Van Winson L, Willemze R, Kanhai HM, Falkenburg JH. Influence of delivery on numbers of leukocytes, leukocyte subpopulation and hematopoietic progenitor cells in human umbilical cord blood. *Blood cells* 1994; 20: 547-59.

Motley D, Meyer MP, King RA, Naus GJ. Determination of lymphocyte immunophenotypic values for normal full term cord blood. *Am J Clin Pathol* 1996; 105: 38-43.

Nakahata T, Ogawa M. Hematopoietic colony forming cells in umbilical cord blood with extensive capability to generate mono and multipotential hematopoietic progenitors. *J Clin Invest* 1982; 70: 1324-8.

Payne TA, Traycoff CM, Laver J, Xu F, Srour EF, Abbound MR. Phenotypic analysis of early hematopoietic progenitors in cord blood and determination of their correlation with clonogenic progenitors: Relevance to cord blood stem cell transplantation. *Bone Marrow Transplant* 1995; 15: 187-92.

Russell ARB, Davies EG, Scopes GID, Daly S, Gordon-Smith EC. Plasma granulocyte colony stimulating factor concentrations (G- CSF) in the early neonatal period. *Br J Haematol* 1993; 86: 642-4.

Saito S, Motoyoshi K, Saito M, *et al.* Localization and production of human macrophage colony stimulating factor (h M- CSF) in human placental and decidual tissues lymphokine. *Cytokine Res* 1993; 12: 101-7.

Shimoda K, Okamura S, Harada N, Omori F, Niho Y. Serum granulocyte colony stimulating factor levels in umbilical cord blood of normal full- term neonates. *Biomed Pharmacother* 1992; 46: 337-41.

Sutherland DR, Keating A, Nayar R, Anania S, Stewart AK. Sensitive detection and enumeration of CD34+ cells in peripheral and cord blood by flow cytometry. *Exp Hematol* 1994; 22: 1003-10.

Vilmer E, Sterkers G, Rahimy C, *et al.* HLA mismatched cord blood transplantation in a patient with advanced leukemia. *Transplantation* 1992; 53: 1155-7.

Wagner JE, Kernan NA, Broxmeyer HE, Gluckman E. Allogeneic umbilical cord blood transplantation:Report of results in 26 patients [Abstract no. 330]. *Blood* 1993; 82 (suppl 1): 86.

Yuen E, Brown RD, Kronenberg H, Richard KA. Human bone marrow progenitor cells stimulated by cord blood. *Exp Hematol* 1986; 14: 771-5.