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FETAL CORD BLOOD'S POTENTIAL FOR BONE MARROW TRANSPLANTATION

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Summary

Approximately 18 years ago, the authors were able to produce an apparently successful bone marrow transplant by using umbilical cord blood. In view of the Chernobyl disaster and the subsequent problems of treatment with marrow transplantation, this study undertook to explore further the potential use of umbilical cord blood as a source of marrow cells. Specimens of umbilical cord blood were collected from 13 routine obstetrical deliveries. All specimens grew erythroid and granulocytic-monocytic colonies. The formation of these various hematopoietic colonies from umbilical cord blood was at least equivalent to bone marrow, and in some instances over 5 times more effective. There appeared to be a statistically significant correlation between the numbers of colony-forming units (CFU-E) and the male infants. The weight of the infants also showed a statistically significant correlation with the burst forming units, erythroid (BFU-E) and the granulocytic-monocytic colony (CFU-GM). The BFU-E also appeared to be greater in number when the time between collection and plating was shorter.

In or around 1965 one of the authors (M.E.) administered umbilical cord blood to several patients in terminal condition and obtained some temporary subjective improvement. One of these, a case of lymphangiosarcoma, was reported in 1966 (1). It occurred to one of the authors (N.E.) at that time that these immunosuppressed terminal patients might be obtaining a temporary bone marrow graft from these transfusions. One case of acute lymphoblastic leukemia, with the technical methods then in existence, obtained what appeared to be a successful marrow graft. The patient underwent temporary remission (2). At that time it was suggested that this could be a source of hematopoietic stem cells readily available for transplantation (2).

Following the nuclear disaster at Chernobyl and attempts to transplant marrow utilizing both bone marrow and, because of a lack of suitable donors, fetal livers (3,4), we undertook to explore further the promise of the earlier study described above. This report is an attempt to determine the extent to which cord blood collected under conditions of routine labor and delivery are able to generate hematopoietic progenitor cells "in vitro", thus providing some measure of its potential for use for marrow transplantation, both in crisis situations, such as Chernobyl, as well as in routine use.

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Methods

Thirteen (13) routine obstetrical delivery cases were utilized in the study. The mothers had been previously tested and found to be negative for hepatitis (HBs/Ag negative), had no known risk factors for acquired immune deficiency syndrome and were routinely seen in the Outpatient Prenatal Clinic of the University Hospital. Upon delivery of each baby, the cord was clamped and cut, and a specimen routinely collected for possible type and crossmatch. The clamp was then released and a 5-20cc specimen of cord blood was collected in a sterile culture media for use in this study.

Cord blood ranging from 5 to 24 ml was directly collected into 1 ml Isocove's modification of Dulbecco's medium (IMDM) (Gibco Labs), and 0.5 ml preservative-free heparin (Forest Pharmaceuticals) containing 500U. Mononuclear cells were separated by Percol (Pharmacia Fine Chemical) sedimentation followed by two washes in IMDM-2% fetal calf serum. The cells were plated at a concentration of 1 x 10^5/ml in IMDM containing 0.8% methylcellulose, 30% fetal calf serum, 1% bovine serum albumin, 1 U/ml r-erythropoietin (a gift fron Genetics Institute, Boston) and 10% phytohemagglutin-stimulated leukocyte condition media (5). Groups of cells under twenty (20) were considered clusters, colonies contained twenty or more cells. Colonies were identified by their characteristic morphology, and enumerated as follows: on day 7 colony-forming units-erythroid (CFU-E), day 10 colony-forming units-granulocytic, monocytic (CFU-GM), and day 14 burst-forming units-erythroid (BFU-E) (6).

The estimate of the number of colonies formed per ml of umbilical cord blood was based on the number of mononuclear cells calculated per ml of cord blood.

Controls were bone marrow obtained for diagnostic purposes on the hematology service and later considered to be normal.

Statistics

Correlations between continuous variables were examined by linear regression models. A student's T-test was used to examine for differences in values when the class variable "sex" was examined. In all calculations, a p value of < 0.5 was considered statistically significant.

Results

All cord blood plated grew erythroid (burst-forming units, BFU-E and colony-forming units, CFU-E) and granulocytic-monocytic (CFU-GM) colonies.

The erythroid colonies per milliliter of cord blood is estimated to vary from 1,968 to 26,568; the granulocytic-monocytic colonies ranged from 1,197 to 15,775 and the burst-forming units varied from 1,968 to 26,564.

There was no evidence of bacterial or fungus contamination in any of the cultures of the cord blood up to 21 days after plating.

There appeared to be no correlation of the number of colony-forming units with the estimated length of gestation or age of mother. The mean colony number (CFU-E) per ml of cord blood was 14230 for males (S.D. + 9380) and 4200 for females (S.D. + 3361). This difference was statistically significant ($p = 0.026$). There was an inverse correlation in the time between collection and plating and the number of BFU-E/ml of cord blood ($p = 0.019$). The weight of the infant was correlated with the BFU-E and the

TABLE I

Sex	Hours*** After Collection	Weight lb oz.	# of Colonies per 1 X 10^5 Mononuclear Cells BFU-E	CFU-E	CFU-GM	# of Colonies/ml Cord Blood BFU-E	CFU-E	CFU-GM
F	3	7.15	179	58	80	5,370	1,740	2,400
F	21	5.30	168	118	63	3,192	2,242	1,197
F	24	6.65	91	78	94	3,367	2,886	3,478
F*	19	7.14	123	238	150	4,059	7,854	4,950
F	28	5.13	64	173	27	3,840	1,380	1,620
M	20	4.14	48	176	107	1,968	7,216	4,387
M	24	4.80	102	516	107	4,896	27,108	5,136
M	6	7.13	415	460	114	9,130	10,120	2,508
M	10	8.13	435	817	214	10,875	20,425	5,350
F	24	7.13	430	593	208	7,274	10,032	3,519
F	12	7.10	278	150	122	6,051	3,265	3,744
M	22	8.60	318	258	190	2,399	1,947	1,434
M	3	8.11	352	246	209	26,568	18,568	15,775
		Mean	231	302	133	6,845	8,829	4,269
		S.E.	±40	±65	±17	±1,796	±2,311	±1,036
Controls** --		Mean	75	384	334			
		S.E.	±10.5	±18	±18.5			

*Caesarian section

**5 Non-leukemic cases who underwent bone marrow aspiration for diagnostic purposes.

***The hours represent the approximate lapsed time between collection and plating.

S.E. - Standard Error

CFU-GM colonies per 1 x 10^5 monocytes and was significant with a p value of 0.0035 and 0.0064, respectively.

Discussion

Considerable knowledge has been gained about the hematopoietic potential of cord blood since our 1972 utilization of cord blood in a marrow transplant. Suppressor activity (7), erythropoietin (8), and human hematopoietic colony-forming cells (9) with extensive ability to generate progenitors for secondary colonies (10,11) have all been described as present in human cord blood. Multi-potential progenitor cells in human cord blood appear to be far easier to demonstrate than in human bone marrow or peripheral blood. According to the authors, 100% of the primary colonies obtained from cord blood had the ability to generate secondary colonies (10). The same laboratory (11) also estimated that there was one blast cell forming colony cell per 5 x 10^4 non-adherent mononuclear cells in cord blood and 1-2 blast cells colony-forming cells per 10^6 non-adherent mononuclear cells in bone marrow. The total number of white blood cells per ml of blood of a newborn at birth has a mean value of 18,100 cells with a mean value of 5.8% monocytes and 7% nucleated red cells per 100 white cells (12).

In our current studies all samples of cord blood plated for colony growth revealed the presence of erythropoietic colonies and granulocytic-monocytic colonies. From our own laboratory, the colony-forming ability of the normal individual's bone marrow is quite similar to that of cord blood (per 1 x 10^5 mononuclear cells). The most noteworthy exception to this similarity is that the erythroid burst-forming units averaged 3 times greater in cord blood (Table 1) than in normal bone marrow. From the literature, one can make estimates that cord blood may well possess as much as 10 or more times the colony-forming ability of bone marrow (13,14,15,16). Two factors which might raise significantly the erythroid colony counts per 1 x 10^5

mononuclear cells would be the higher counts found in male infants' cord blood (p value 0.022) and the length of time between collection of the cord blood until plating, as the burst-forming erythroid units appeared to have an inverse relationship to lapsed time by the technique used herein (p value 0.019). From previous studies we have found one can usually recover from 25 to 100 ml of cord blood per delivery.

These cord blood samples were collected during routine deliveries and showed no evidence of contamination; this lack of cord blood contamination was also noted in our earlier studies when the blood was cultured for bacteria and fungus (1,2).

Since there appears to be a direct correlation between the number of transfused colony-forming cells and a successful marrow transplant (16), cord blood with its relatively high numbers of colony-forming cells appears to be an ideal source of cells for bone marrow transplantation. The current ability to freeze marrow successfully for transplantation (16,17,18) could make cord blood, now a waste product, a readily available source of primitive hematopoietic cells which potentially could be utilized in marrow transplant and particularly where a suitable donor is not available. If the number of bone marrow transplantations continues to increase as it has in recent years for multiple reasons (19), including what currently is considered experimental, umbilical cord blood could potentially serve as an unlimited donor source.

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