Transient spontaneous engraftment of CD34 hematopoietic cord blood stem cells as seen in peripheral blood: treatment of leprosy patients with anemia by placental umbilical cord whole blood transfusion

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Summary

Cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and white blood cell (WBC) counts, and a plasma filled with cytokine and growth factors, as well as its hypoantigenic nature and altered metabolic profile, has all the potential of a real and safe alternative to adult blood transfusion.

Our experience of 74 units (50 ml - 146 ml mean, 86 ml ± 7.6 ml SD, median 80 ml, mean packed cell volume 48 ± 4.1 SD, mean percent hemoglobin concentration 16.2 g/dl ± 1.8 g/dl of placental umbilical cord whole blood collection (from 1 April 1999) after lower uterine cesarean section (LUCS) from consenting mothers and transfusion of the same to 16 informed, consenting patients with percent plasma hemoglobin 8 g/dl or less, is presented here. After collection the blood was immediately preserved in the refrigerator and transfused within 72 hours of collection. Fifteen males and one female, aged 12-72 yrs (mean 48.4 yrs) participated: five cases were pausibacillary type (PB) and 11 cases were multibacillary type (MB). The clinical spectrum of the cases varied widely from the tuberculoid to the lepromatous type and one patient presented with gangrene of the leg preceding an auto amputation which was infested with maggots. Each case was approved by the institutional ethical committee and received two to eight units of freshly collected placental umbilical cord blood in one transfusion without encountering any clinical, immunological or non-immunological reaction. Seven days after completion of the placental umbilical cord blood transfusion, the peripheral blood hematopoietic stem cell (CD34) estimation revealed a rise from the pretransfusion base level (.09%), varying from 3.6% to 16.2%, in 75% of the cases, without provoking any clinical graft vs host reaction in any of the leprosy victims. This value returned to normal within three months in most cases.

Key words: Safe placental umbilical cord blood transfusion; Immune mosaic leprosy transient transplantation; Impact of transfusion.

Introduction

Leprosy is a chronic infectious disease caused by acid-fast mycobacterium known as mycobacterium leprae. It affects the skin and the peripheral nerves, and can also affect the eyes, upper respiratory tract, etc. In the year 1873, the famous Norwegian physician, Gerhard Henrik Armauer Hansen, first identified the organism. The word leprosy comes from the Latin word lepros, which means defilement. This disease has been documented since antiquity. Leprosy was considered a divine curse for sin in the Old Testament and is also documented in Hindu and Buddhist texts as the result of Karma, i.e., reaction for committing injustice or crimes against mankind. Earlier, in the lay mind, the disease was deemed incurable due to its slow spread and because it caused severe deformity, disability and social stigmatization. Leprosy was endemic in Western Europe since the medieval period. It was eliminated in Scandinavian countries as recently as the early 20th century as a result of the improved socioeconomic index: the people were provided with better housing, nutrition, clean water supply and there was an overall improvement in living standards.

In 1991, the World Health Organization (WHO) and its member states committed themselves to eliminating leprosy as a public health problem by the year 2000. While leprosy is still a major problem in most developing countries, India and Brazil are among the top six countries in the world with a prevalence rate of 4.3 to 4.5 per 10,000 persons. India alone accounted for 78% of the 690,830 newly detected cases in 2001 [1-3].

The major goals of the leprosy control program are early detection, proper treatment and adequate care for prevention of disabilities, and rehabilitation. There are several effective chemotherapeutic agents against M. leprae, of which dapsone (diaphenylsulfone, DDS), rifampicin, clofazimine, ofloxacin and minocycline constitute the backbone of the multidrug therapy as recommended by the WHO. Gastrointestinal toxicity and skin discoloration are the major side-effects for long-term treatment with clofazimine, and rifampicin is known for its hepatotoxicity. However, the most important side-

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effect, which is prominent, is the hematological problem with dapsone [4]. The common hematological side-effects with dapsone therapy are hemolytic anemia, methmoglobinemia, reticulocytosis, and reduction of cell resistance as seen in osmotic fragility studies. These effects are much more pronounced in lepromatous leprosy patients with blunted erythropoietin response and low serum iron together with mildly raised serum ferritin concentrations. The problem of dapsone therapy is complicated further in endemic areas where, because of low nutrition, malaria and intestinal parasitism, the hemoglobin concentration is already compromised.

In order to combat anemia in leprosy patients, with varying degrees of refractoriness due to drug on the disease or the host reaction [5-10], Dharmendra [11], a noted leprosy specialist from India, suggested many years back, blood transfusions from other consenting leprosy patients. In India, the placenta is a readily available source of fresh whole blood, rich in fetal hemoglobin, which can carry more oxygen than adult hemoglobin.

This placenta or the afterbirth is discarded routinely everywhere in the world and (in India alone, there are more than 20 million placentae produced as afterbirth every year) is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated or incinerated. The centers of excellence in the Western developed world have been working on the use of a tiny microscopic fraction of cord blood, i.e., CD34 stem cells only (.01% of the nucleated cells of the placental blood). My team of doctors has been successfully transfusing placental cord whole blood as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology [12, 13].

Whether fetal hemoglobin rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue vol/vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term), could be an emergency and safe substitute for adult whole blood in cases of leprosy victims with percent hemoglobin concentrations of less than 8 g/dl, was the main idea behind the present study.

Materials and Methods

Seventy-four units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine cesarean section (LUCS) under general or regional anesthesia. If there was gross prematurity or dysmaturity, or the projected weight of the fetus was less than 2 kg, or there was any specific disease of the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed consenting, healthy mothers after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the mother's stable physical condition. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14 ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4°C, after putting the necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, and malaria as per standard blood transfusion protocol (reported on earlier) [14]. The collected cord whole blood was transfused within three days of collection to a leprosy victim with anemia, after grouping, cross-matching and following the standard adult blood transfusion WHO guidelines for transfusion, and strictly adhering to the institutional ethical committee guidelines and the patient consent protocol. Pretransfusion and seven days after the transfusion, blood was drawn from which consenting patients for peripheral blood hematopoietic stem cell estimation (CD34) was done by flow analysis cytometry as per standard protocol at Ranbaxy laboratory, and which was repeated after three months for comparison.

Results and Analysis

Leprosy is almost exclusively a disease of the developing world with 80% of its victims residing in India, China, Indonesia, Myanmar, Brazil, Nigeria, etc. The disease is practically absent in the USA (less than 200 cases detected in outsiders per year). Poverty, rural background and its impact on socioeconomic, educational and nutritional status are very important for the long incubation and transmission of the disease.

In Calcutta, we are working on the problem of anemia in leprosy victims undergoing treatment. Varying degrees of anemia are very prevalent in leprosy patients, which could be due to background malnutrition, coexistent diseases like helminthiasis or drug impact on the immune system (including bone marrow), poor red cell survival, and rarely Glucose-6-phosphate dehydrogenase deficiency (dapsone therapy). Thus far, 16 cases (15 males and one female, aged 12-72 yrs, mean 48.4 yrs) have been treated. Five cases were pausibacillary type (PB) and 11 cases were multibacillary type (MB). The clinical spectrum varied widely from the tuberculoid to the lepromatous type and one patient presented with gangrene of the leg preceding an auto amputation and was infested with maggots (Figure 1). MB patients received 600 mg rifampicin once monthly and 200 mg clofazimine initially, followed by 50 mg daily, along with 100 mg dapsone daily for 12 months uninterrupted. PB patients received 600 mg rifampicin once monthly along with 100 mg dapsone daily for six months. In the present series we collected 74 units of placental umbilical cord whole blood after lower uterine cesarean section (LUCS) from consenting mothers and transfused the blood (vol 52-142 ml, mean 83 ml and ± 14 ml SD) to leprosy patients with anemia within three days of collection (the blood was stored in a refrigerator until used). We followed the standard safe transfusion protocol as per WHO guidelines, and transfused two to eight units of placental blood to each patient without encountering any immunological or non-immunological reactions. No immediate reactions

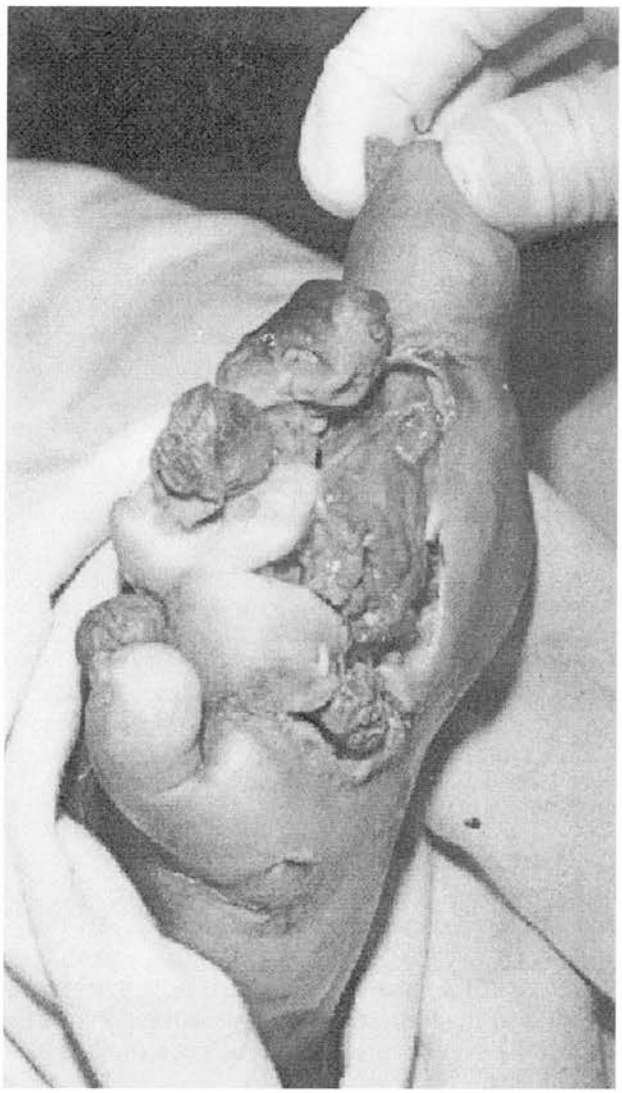

Figure 1. — Photograph showing an infected gangrene and ulceration which was studded with maggots, in the background of leprosy.

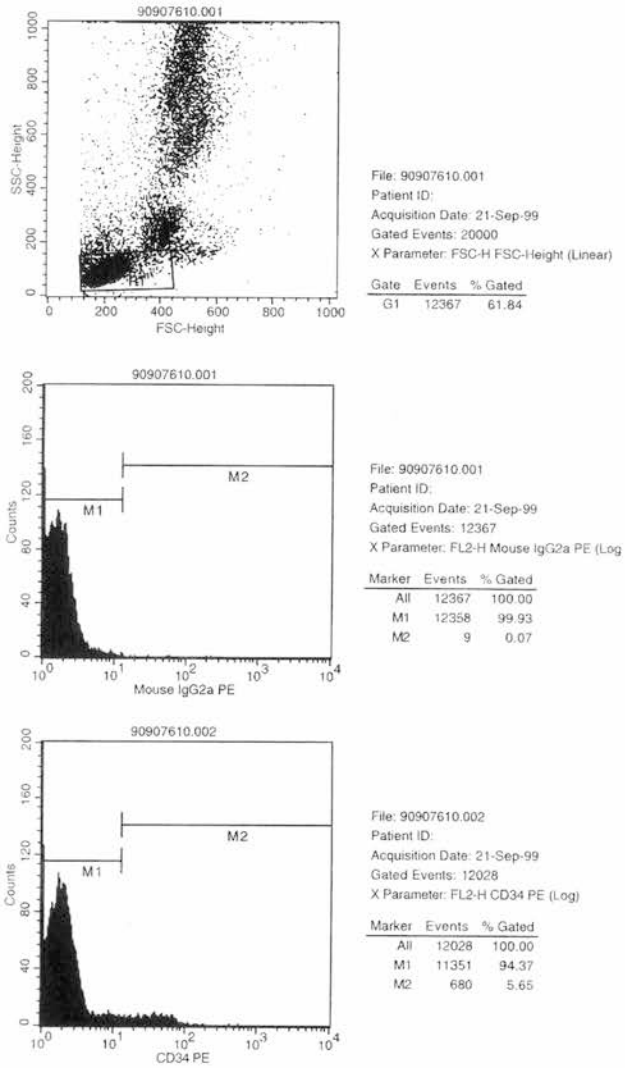

Figure 2. — Flow analysis study showing transient CD34 peripheral blood level rise after cord blood transfusion.

due to transfusion, viz., fever, chill and rigor, flank pain, back pain, blood in urine, fainting or dizziness were seen in any of the cases. Even late reactions like mild or progressive renal complications were not encountered. Feto-maternal cell traffic has been implicated for the cause of scleroderma in mothers in cases of male babies. In the present series, we did not see any such rare or unusual complication due to neonatal blood transfusion in the adult system.

In some leprosy patients, seven days after the completion of the cord blood transfusion, the flow analysis cytometry study showed a rise (precord blood transfusion peripheral blood CD34 normal range is up to .09%) of peripheral blood CD34 level, from the pretransfusion level, varying from 3.6% to 16.2%, in 75% of the cases (one case where the FACS showed 5.65% peripheral blood CD34 is shown in Figure 2). This effect became normal after three months. A question may be raised - why was there peripheralization of the CD34 cells? There was no clinical sign of graft vs host reaction in any of the patients. There was no growth factor or any other bone marrow stimulating or suppressing drug utilized during the transfusion protocol for the cord blood.

Discussion

The possible reason behind the transient rise in hematopoietic stem cells as seen in the peripheral blood in HLA-randomized recipients without any immunosuppressive support and without provoking clinical graft vs host reaction still remains a mystery. However, probable explanations may be that the placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in umbilical cord blood (UCB) transplantation. Besides intrinsic differences, hematopoietic stem cell (HSC) sources in umbilical cord

blood (UCB) cells have had a different set of microenvironmental exposures compared to those of adult marrow or peripheral blood stem (PBS) cells. All HSC sources are influenced by the microenvironment from which they are derived. An example of differences between sources are some of the observed changes in HSC cycle status, gene expression and adhesive and invasive properties induced by mobilization procedures used to generate PBS cells, e.g., G-CSF. The placenta is a complex organ that regulates fetomaternal interactions. Many cytokines that can influence lymphohematopoietic development, e.g., granulocyte colony stimulation factor (G-CSF), c-kit ligand (stem cell factor [SCF]), granulocyte macrophage colony stimulating factor (GM-CSF), Interleukin 15 (IL-15), and others are produced by the placenta. Production of G-CSF by the placenta may be especially relevant to UCB transplatation. G-CSF is produced both by the maternal decidua and the fetal chorionic villi, and enters the fetal circulation by a process that does not require a functional G-CSF receptor. G-CSF from the mother probably does not enter the fetal circulation as administration of recombinant human G-CSF (rhG-CSF) to pregnant macaques did not result in detectable rhG-CSF in the fetuses [15]. The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother and fetus from each other's allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogeneic targets including choriocarcinoma cells.

Finally, though precisely not clear as yet, the functional hypoantigenecity of the cord blood antigen with its complex cytokine interaction may have a role in immune selective-masking in leprosy, i.e., immune mosaicism in those anemic patients with leprosy either due to dapsone-like drugs, disease, nutrition or helminthiasis, or other associated factors like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient. All the patients irrespective of their background tolerated the procedure well and there was a sense of well being in most cases.

Our group of researchers has been working on the problem of fetal cell or tissue transplant in the adult system as well as the use of umbilical cord whole blood transfusion as an alternative to adult whole blood transfusion from the pediatric to the geriatric age group for different indications since 1 April 1999 [16-20]. We are of the opinion that growth factor and cytokine-filled cord blood collected aseptically from the placenta of consenting mothers after the birth of a healthy baby, has all the potential of an effective therapeutic adjuvant for leprosy patients with anemia in the underprivileged world. Cord blood is protected from infection as a result of nature's finest biological sieve, i.e., the placenta, and contains 60-80% fetal hemoglobin (which can carry 60% more oxygen than adult hemoglobin), and moreover, has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential to convert TH2 responses (lepromatous) to TH1 responses (tuberculoid), due to its rich cytokine and growth factor content, which may have a role in immune response modification.

Conclusion

UCB is now accepted as an alternative source for hematopoietic stem cells for transplantation especially in children. Unrelated UCB offers many practical advantages as an alternative source of stem cells, including: 1) easy availability without risk for mothers and donors; 2) less possibility of infection, particularly cytomegalovirus; 3) documented reduced risk of GVHD, with easy HLA-matching criteria for donor-recipient selection; and 4) UCB banks have been established for related and unrelated UCB transplantation with about 100,000 units currently available [21].

In this preliminary communication and follow-up for six years, we have seen that cord blood transfusion is safe in leprosy patients with anemia. However, the unique phenomenon of transient spontaneous transplant impact of the CD34 cells, as seen in peripheral blood after cord blood transfusions in HLA-randomized recipients without the support of immunosuppressives and without provoking clinical graft vs host reaction, has to our knowledge never been cited in published medical literature.

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