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PARKINSON'S DISEASE MICE AND HUMAN UMBILICAL CORD BLOOD

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Subjects: Mice with Weaver mouse spontaneous mutation
Abbreviation: HUCB= Human Umbilical Cord Blood

Abstract

In 1995, it was suggested that immature stem cells (Berashis Cells) existing in human cord blood might have an ameliorating effect on such neurological diseases as Alzheimer's, amyotrophic lateral sclerosis and Parkinson's disease. Since these predictions, we have been able to successfully extend the length of life of mice with amyotrophic lateral sclerosis [B6SJL-TgN(SOD1-G93A)IGUR], Huntington's Disease (B6CBA-TgN(H.Dexon1)62Gpb and Alzheimer's mice [Tg(HuAPP695.SWE)2576]. Recently we expanded the studies to include mice with Parkinson's Disease. 32 mice, 6-12 weeks old B6CBACa-AW-J/A-Kcnj6<wv> were obtained from Jackson Laboratory, Bar Harbor, Maine. The mice were divided into 3 groups:

(A) 10 untreated control mice, (B) 10 mice treated with 5.6 x 10^6 congenic bone marrow mononuclear cells and (C) 12 mice receiving 100-110x10^6 HUCB mononuclear cells intravenously. No immunosuppression was used.

When 50% of the controls were dead only 1 of the 10 mice receiving congenic marrow and 2 out of 12 mice that received cord blood mononuclear cells were dead. This preliminary study was terminated when the animal's were 200 days old, at that time one out of 10 controls was alive. Out of 10 mice that received congenic bone marrow, 2 were alive. Out of 12 mice that received megadoses of cord blood mononuclear cells 4 were alive. Survival curve of mice that had congenic marrow had a p value of <.05; the survival curve of mice receiving cord blood mononuclear cells had a p value <.001 (Fig 1) compared to controls.

Human umbilical cord blood mononuclear cells significantly delayed the onset of symptoms and death of Parkinson's disease mice. This effect was greater than that produced by congenic bone marrow cells.

Introduction

The possibility that human embryonic stem cells might improve the clinical status of patients with Parkinson's disease has recently received considerable attention. In addition, various stem cells including cells derived from the mesencephalon of embryos have been implanted into the brains of patients with severe Parkinson's disease with clinical benefits in younger but not in older patients (Freed, Greene *et al.* 2001)

Several years ago it was suggested that primitive cells, which we call "Berashis Cells", that exist in human umbilical cord blood in small numbers, might also have an ameliorating effect on such neurological diseases as amyotrophic lateral sclerosis, Alzheimer's disease, and Parkinson's disease (Ende, 1995, 2000). Since these early predictions we have been able to successfully delay the onset of symptoms, and extend the length of life of transgenic mice with amyotrophic lateral sclerosis B6SJL:-TgN(SOD1-G93A, GUR), Huntington's Disease mice (B6CBA-TgN(HDexon1)62Gpb and Alzheimer's mice (Tg(HuAPP695.SWE)2576. (Chen and Ende 2001; Ende and Chen, 2001; Ende and Chen *et al.* 2001). The studies on SOD_1 mice (A.L.S.) have been recently confirmed and presented at the 12th International

Symposium on ALS/MND, Oakland USA, November 2001 (Kalkanis, Drelbelbis *et al.* 2001). Recently we expanded the studies to include mice with Parkinson's disease.

In all previous studies, as part of the controls, we always included both untreated mice and mice that received bone marrow cells from appropriate congenic animals. SOD1 mice treated with congenic bone marrow cells showed some improvement over untreated controls (Chen and Ende, 2000). Alzheimer's and Huntington's mice did not. It is the unusual effect of both the mouse congenic marrow cells and human umbilical cord blood cells on the survival of mice with Parkinson's disease that this report is directed. It is understood that additional clinical and pathological studies are necessary to more fully evaluate these findings.

Materials and Methods

Cord Blood and Storage

Human umbilical cord blood samples (HUCB) were obtained from the placentas of healthy full-term neonates. Each cord blood sample was collected into a 50 mL sterile polypropylene test tube containing 5 mL of citrate phosphate dextrose as an anticoagulant. The volume of the HUCB collected varied from 20 mL to 40 mL. Samples were kept at room temperature and within 24 hours they were sent to the blood bank. The HUCB samples were then transferred into a special polyolefin blood collection bag (Cryocyte Freezing Container, Baxter Healthcare, Deerfield, IL) that allow gaseous transfer and stored at 4°C in a blood bank refrigerator for 10 to 13 days (Lemoli, Tafuri *et al.* 1992). Specimens from another institution were not transferred for 48-72 hours. The donor specimens when available were combined 3-4 days before administration according to their blood type (ABO). Frequently, however, only one donor was available. After storage at 4°C the HUCB units were transferred to 15 mL disposable centrifuge tubes and the mononuclear cells (MNC) were separated from the whole cord blood by centrifugation for 30 minutes at 1700 RPMs with ficol histopaque (Simga, St. Louis, MO). Portions of the cord blood mononuclear cells were removed to provide the desired number of cells (MNC) per mouse. The MNC cells were then washed twice with phosphate buffered saline (PBS) and centrifuged for 10 minutes at 1,000 RPMs. One mL of PBS

was added to the pellet for counting. After the viability counting was determined, the mononuclear cells were centrifuge for 10 minutes at 1,000 RPMs. 0.2 mL of PBS solution was added for final dilution and injection intravenously (retro-orbital). This process was repeated the next day to bring the total count of HUCB mononuclear cells up to desired number.

Animals

Thirty-two animals 6-12 weeks of age, (B6CBACa-AW-J/A-K cnj6 <wv>) were received from Jackson Laboratory Bar Harbor, Maine. These mice are homozygous for the weaver spontaneous mutation. They are recognizable in the second postnatal week by small size, instability of gait, weakness and hypotonia (Jackson Laboratory). Injection of monocular cells began 2 weeks after the animals were received. The animals were divided into three groups. (A) A control group of 10 mice that received no treatment. (B) Ten (10) mice that received 5.6 x 10^6) monocular bone marrow cells from congenic mice and (C) twelve (12) mice that received 100-110 x 10^6) (megadose) cord blood monocular cells in two doses of 50-60 x 10^6 cells each given approximately 24 hrs apart. The animals where euthanized when they became unable to feed or drink. Decision to euthanize was made by the animal handlers who were unfamiliar with the treatment administered.

Results

When 50% of the controls were dead only one of the 10 mice receiving congenic marrow and 2 out of 12 mice the received cord blood mononuclear cells were dead. This preliminary study was terminated when the animals were 200 days old and at that time only one out of 10 controls was alive. Out of 10 mice that received congenic bone marrow 2 were alive. Out of 12 mice that received megadoses of cord blood mononuclear cells 4 were alive. The survival curve of mice that had congenic marrow cells had a p value of <0.05 and the survival curve of mice with cord blood mononuclear cells had a p value of <0.001 (Fig 1). All mice surviving to 200 days had their identification confirmed by DNA analysis of tail clips by Jackson laboratory. A cusory search of the literature, however, failed to show any examples of where bone marrow transplantation has been used therapeutically on patients with Parkinson's disease.

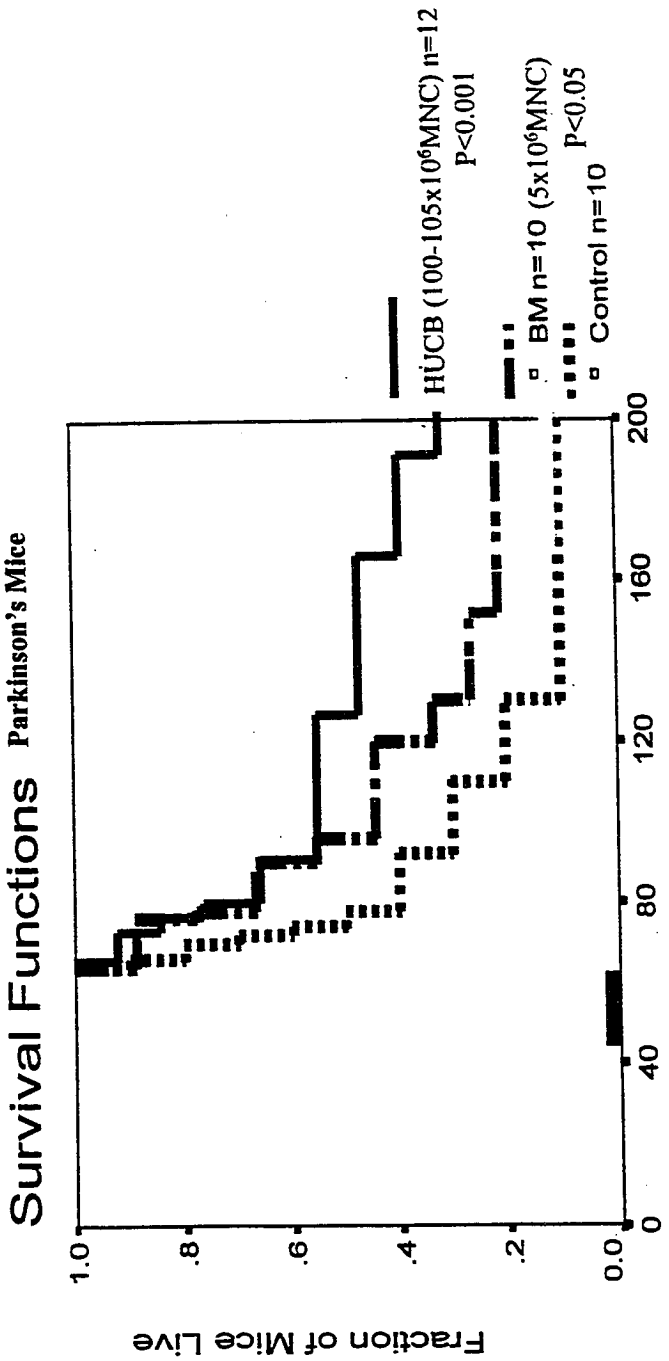

Figure 1

Days of transplantation

HUCB: Human Umbilical Cord Blood
MNC: Mononuclear Cell
BM: Bone Marrow

Discussion

For a number of years we have felt that human umbilical cord blood contained very immature cells. These cells are relatively few in numbers with similar potential to embryonic stem cells (Ende, 1995; Ende 2000) which for identification purposes we have call "Berashis Cells". To increase the number of these Berashis Cells, we have utilized megadoses of human cord blood mononuclear cells to treat various animal models. By this method we have succeeded in delaying the onset of symptoms and increasing the life span of animal models of amyotrophic lateral sclerosis (Chen and Ende, 2000), Huntington's disease (Ende and Chen, 2001) and Alzheimer's disease (Ende, Chen *et al*, 2001). In this current study by utilizing megadoses of cord blood mononuclear cells we have also succeeded in increasing the life span of Parkinson's disease mice.

As noted in the result section, when compared to untreated controls, there was a significant increase in survival of mice receiving congenic bone marrow ($P<0.05$) and a further increase in survival of the animals receiving megadoses of human cord blood mononuclear cells ($p <0.001$). Finding of an increase in survival of animals receiving congenic marrow cells in doses adequate to produce an Isograft has been noted in some of our previous experiments on amyotrophic lateral sclerosis mice (Chen and Ende, 2000). A search of the literature, however, failed to show any examples of where bone marrow transplantation has been used therapeutically on patients with Parkinson's disease.

There is a recent publication where the authors have found that a single cell that homes to the bone marrow has the ability to differentiate into cells of the G.I. tract, lung and skin (Krause, Theise *et al.* 2001) The authors state that "It is possible that the cells are summoned to sites of injury by factors secreted from damaged injury". (Krasue, Theise, *et al.* 2001) This raises the possibility of a dormant stem cell (Berashis cell) of the umbilical cord blood may home to dying cells. We have been able to detect trace evidence of human DNA in mice12 month after they received cord blood mononuclear cells (Ende, Ponzio *et al.* 1999), which would indicate that the "Berashis Cell" might persist for an indefinite time period. In addition, recent related studies have shown that bone marrow cells can be converted to neurogenic cells and neurogenic cells can be converted to bone marrow cells (Buomson, Rietze, *et al* 1993 Eglitis and Mezey, 1977).

In conclusion, if these immature cells in human cord blood (Berashis cells) which seem to have similar potential functional properties to embryonic stem cells (Ende 2000), can be given intravenously by means of megadoses of cord blood mononuclear cells similar to the animal model described here in, a potential therapeutic agent may be available to patients with Parkinson's disease. The success in prolonging the life of the animal model with congenic bone morrow cells may also indicate that marrow cells with HLA compatibility may have a similar capacity as cord blood.

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