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NOD/LtJ TYPE I DIABETES IN MICE AND THE EFFECT OF STEM CELLS (BERASHIS) DERIVED FROM HUMAN UMBILICAL CORD BLOOD

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Abstract

Previously we have successfully delayed the onset of vasculitis and death in MRL Lpr/Lpr mice that are considered to have an autoimmune disease similar to human lupus erythematosus. Likewise, with the use of megadose human umbilical cord blood mononuclear cells, we were able to delay the onset of symptoms and death in SOD1 mice that carry a transgene for amyotrophic lateral sclerosis, considered by some to be an autoimune disease. A similar approach was utilized with NOD/LtJ type 1 diabetic mice. By administering megadoses of human umbilical cord blood mononuclear cells we were able to ameliorate the disease and improved the life span. This occurred to a greater extent than with bone marrow obtained from congenic mice. No immunosuppression was utilized in this study. This study raises the possibility of utilizing human

cord blood mononuclear cells in conjunction with pancreatic islet transplantation.

Introduction

In 1995 we reported that MRL Lpr/Lpr mice, considered to have an autoimmune disease similar to lupus erythematosus, had a considerable delay in both the onset of vasculitis and death when given human cord blood mononuclear cells and sublethal irradiation (Ende, Czarneski, et al.,1995). Although controversial, amyotrophic lateral sclerosis is also considered an autoimmune disease (Rowland, 1992). Recent studies on SOD1 mice, that carry a human transgene associated with amyotrophic lateral sclerosis, have shown a delay in the onset of symptoms and death when given megadoses of human cord blood mononuclear cells even without the use of immune suppression (Ende, Weinstein, et al., 2000) (Ende, 2000). Type 1 diabetes mellitus, is a chronic dysmetabolic disease of multifactorial pathogenesis and also is generally considered to be an autoimmune disease (Hua, Ricordi, et al., 1999). This study was undertaken to determine if megadoses of human cord blood mononuclear cells, without the use of immunosuppression, would have an ameliorating effect on type 1 diabetic mice. This is a report of the preliminary findings of this study.

Materials and Methods

The study was approved by the Institutional Review Board of New Jersey Medical School, Newark, N.J. The mice were housed in an AAALAC-1 approved animal laboratory. The project was approved by the institutional Animal Review Committee.

Collection and Preparation of Cord Blood

Human umbilical cord blood samples were obtained from 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 collected varied from 20 to 40 ml. and the samples were kept at room temperature until they were sent to the blood bank for storage. The samples were then transferred into a polyolefin blood collection bag (Cryocyte Freezing Container, Baxter Healthcare, Deerfield, IL) that allows gaseous transfer and were stored in 4 ˚C blood bank refrigerator. Donor

specimens were combined according to their blood type (ABO) (Lu, Ende, 1994), (Ende, Lu, et.al., 1999). However, since both the availability of cord blood and the volume required to obtain the desired number of cells varied widely, many of the mice received all the cells from a single donor. After storage for 10-13 days, units were placed in a 15 ml disposable centrifuge tube and the mononuclear cells (MNC) were separated from the whole cord blood by centrifugation for 30 minutes at 1700 RPM with ficol histopaque (Sigma, St. Louis, MO). Portions or all of each stored bag were removed to provide the desired number of mononuclear cells per mouse. The cells were then washed twice with phosphate buffered saline (PBS) and centrifuged for 10 minutes at 1000 RPM. One ml of PBS was added to the pellet for counting. After the viability and counting were determined, the MNC were centrifuged for 10 minutes at 1000 RPM, 0.2 ml of PBS solution was added for final dilution and injection into the mouse (retro-orbital). This process was repeated the next day to bring the total number of mononuclear cells given the animals up to 100 x 10^6.

Animals

The mice, NOD/LtJ stock number 001476, were received from Jackson Laboratory Bar Harbor, Maine. NOD/LtJ mice are characterized by insulitis and marked decrease in pancreatic insulin content which occurs around 12 weeks of age in the female. The onset of diabetes is marked by moderate glycosuria and non-fasting plasma glucose greater than 250 mg/dL. When diabetic, the mice are hypoinsulinemic and hyperglucogonemic. The age of the animals on injection varied from 7 to 9 weeks of age depending on the availability of cord blood. Urine glucose was performed weekly. All blood glucose determinations where made when the animals were sacrificed.

Preparing Bone Marrow for Injection

Bone marrow cells were obtained from wild type mice (NOR/LtJ). After euthanization, the bone marrow was extracted from the femur and tibia by lavage with PBS. The bone marrow cells were prepared and injected in the same manner as the cord blood cells.

Procedure

Thirty female mice were obtained from Jackson Laboratory and divided into three groups of ten mice each. One group was untreated, one group received 5.6 x 10^6 bone marrow mononuclear cells from a NOR/LtJ congenic male donor, and one group of mice received 100 X 10^6 cord blood mononuclear cells intravenously in the retro-orbital venous plexus. 5 X 10^6 bone marrow mononuclear cells are considered adequate for a successful isograft transplant in mice.

Results

The 10 control mice reached serum glucose levels greater than 2000 mg/dL by the 123 day and 6 out of 10 mice of this group were dead by 123 days (Table I). All the remainder animals were sacrificed at 137 days. All blood sugar determinations were done at the time of the sacrifice. The average blood glucose level was lowest (239.7 mg/dL) in the animals that had received cord blood as compared to both the control animals (474.25 mg/dL) and mice receiving 5.6 x 10^6 bone marrow mononuclear cells (358.8 mg/dL) (Table1). The urine glucose levels had a similar pattern to the blood glucose in the surviving mice. At 137 days 8 of the 10 mice that received cord blood were alive and only 4 of 10 control mice were still alive.

Discussion

Since there is evidence that very immature pleuropotential stem cells, probably totepotential, which we call "Berashis Cells," exist in human umbilical cord blood in small numbers, we have attempted to determine if large doses of cord blood mononuclear cells can provide enough of these cells to affect the clinical course as manifested in various mouse animal models of human disease (Ende, 2000). We feel these immature cord blood cells (Berashis Cells) may have similar physiological properties to those attributed to embryonic stem cells. In previous studies significant improvement was obtained in the delay of the onset of illness and prolongation of life in the following animal models: MRL Lpr/Lpr mice, an animal model similar to human lupus mice (Ende, Czarneski, 1995), SOD1 mice that carry a transgenic gene for amyotrophic lateral sclerosis (Ende, Weinstein, 2000), Huntington disease (B6CBA-TgN(Hexon1)62Gpb) (Ende, Chen, 2000) and Alzheimer's disease [Tg(HuApp 695. SWE)2576] mice (Ende, Chen,

Table I

NOD/LtJ diabetic mice treated with mononuclear cells from congenic mouse bone marrow and human cord blood (HUCB)

Control				Bone Marrow ($5.6x10^6$MNC)				HUCB($100x10^6$MNC)			
		Glucose (mg/dL)				Glucose (mg/dL)				Glucose (mg/dL)	
n=10	Days	Urine	blood	n=10	dyas	Urine	blood	n=10	days	Urine	Blood
Died	100	2000		Died	95	1000		Died	95	1000	
Died	102	2000		Died	99	1000		Died	102	800	
Died	103	2000		Died	109	1000					
Died	104	2000		Died	121	1000					
Died	122	2000						Sac	137	200	169
Died	123	2000						Sac	137	300	194
				Sac	137	800	647	Sac	137	300	292
				Sac	137	600	472	Sac	137	400	138
Sac	137	500	474	Sac	137	450	308	Sac	137	400	301
Sac	137	600	482	Sac	137	300	176	Sac	137	500	249
Sac	137	>700	500	Sac	137	300	404	Sac	137	500	403
Sac	137	630	441	Sac	137	200	146	Sac	137	600	172
survival	4 out of 10			6 out of 10				8 out of 10			
Average blood glucose at sacrifice			474.25				358.8				239.7
Average highest urine glucose determination		1443				665				500	

life span	Blood Glucose	Urine Glucose
HUCB vs Controls: p<0.01	HUCB vs Controls: p<0.0001	HUCB vs Controls: p<0.0001
BM vs Controls: p<0.05	HUCB vs BM: p<0.05	HUCB vs BM: p<0.01

et al., 2001). In this publication we describe similar effects on Type I insulin dependant diabetes mellitus mice without the use of immunosuppression. In this study there was a delay in the appearance of elevated blood and urine glucose levels and improvement in survival over non-treated control animals. The finding that congenic bone marrow had a positive effect on the course of the disease in these mice is consistent with previous publications, where infusion of donor bone marrow produce indefinite survival of rat islet allografts (Ricordi, Murase, *et al.*, 1996). Megadoses, however, of human cord blood cells without immonosuppression were able to produce a greater clinical effect on NOD/LtJ mice than congenic bone marrow cells when given in the usual quantity (5×10^6 cells) that would produce a successful isograft in mice. The recent success in treating type I diabetes with pancreatic islets (Shapiro, Lakey, *et al.*, 2000) raises an interesting possibility of simultaneously administering a megadose of human cord blood mononuclear cells with the islets. Perhaps the stem cells found in the umbilical cord blood (Berashis Cells) may have a symbiotic effect on the transplanted islets cells, similar to bone marrow administered simultaneously with islets in rodents (Ricordi, Murase, *et al.*, 1996). Human umbilical cord blood cells would not have to be closely HLA matched and could be probably obtained in large enough quantities for use in an adult (Lu, Ende, 1997), (Ende, Lu, *et al.*,1999).

In conclusion, human umbilical cord blood mononuclear cells without immunosuppression produced a significant amelioration of findings and survival of NOD/LtJ mice which suggests the possibility that human cord blood cells may be useful in the armamentarium to treat type I diabetes.

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