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MURINE SURVIVAL OF LETHAL IRRADIATION WITH THE USE OF HUMAN UMBILICAL CORD BLOOD

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Summary

We have found that human umbilical cord blood (HUCB) will routinely protect mice exposed to lethal levels of irradiation. At the end of 50 days, over seventy percent (70%) of mice injected with HUCB survived 900 cGy or irradiation, which produced 100% deaths in the uninjected control animals. Moreover, there was some evidence that human colony stimulating factors further improved survival. Anti-Natural Killer cell (NK) antibody was utilized along with HUCB in these studies, however, Anti-NK cell serum alone had no radioprotective effect in mice. The studies reported here suggest the possibility of utilizing HUCB for immediate protection of humans from lethal irradiation.

Allogeneic bone marrow has been utilized for many years in attempts to rescue individuals who have been exposed to lethal levels of irradiation (1). Following the Chernobyl disaster, allogeneic human bone marrow and fetal liver cells were given to the victims (2 & 3). Those people injected with fetal liver cells seemed to receive no beneficial effect (3). Bone marrow transplants were believed to be beneficial to some victims, producing a transient engraftment, which allowed recovery of their own marrow (2).

In a preliminary experimental study (4), where SJL/J mice were lethally irradiated with a dose of 900 cGy, we reported the survival of 4 out of 10 mice (5 sacrificed) that received HUCB. This was comparable to the survival of 2 out of 10 mice (5 sacrificed) that received 900 cGy of irradiation and syngeneic bone marrow.

The present study was undertaken to further investigate the above findings using HUCB as an alternative to bone marrow to protect animals from lethal levels of irradiation. We also wished to determine if human hematopoietic growth factors, or cyclosporine, given in conjunction with HUCB, could further improve the survival rate. It is conceivable that if mice can be protected from lethal irradiation with HUCB despite existing major immunological histocompatibility barriers, then perhaps a similar therapeutic approach would be feasible for humans exposed to lethal doses of irradiation, either therapeutically or following a nuclear disaster.

Materials and Methods

SJL/J mice (8-10 weeks old) were obtained from the Jackson Laboratory (Bar Harbor, Maine), and were housed in the AAALAC -accredited Research Animal Facility of the New Jersey Medical School.

Irradiation was delivered from the 8 MeV X-ray beam of a linear accelerator (Philips SL 75-20) using a 25 x 25 cm field with a rate of about 400 Gy/min. Animals were restrained during irradiation in a lucite box measuring 19.8 x 15.9 x 6.5 cm, which holds 10 mice in individual compartments.

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Polystyrene blocks of 5.22 and 5.46 cm thickness were placed above and below the cage, respectively, to provide dose build-up and back scatter. The source surface distance of the irradiation assembly was 89 cm, with a mice a mean distance of 100 cm from the X-ray source. Irradiated animals were housed in a laminar flow compartment with sterilized cages, food, water and bleeding for the duration of the experiment and given antibiotics in their drinking water.

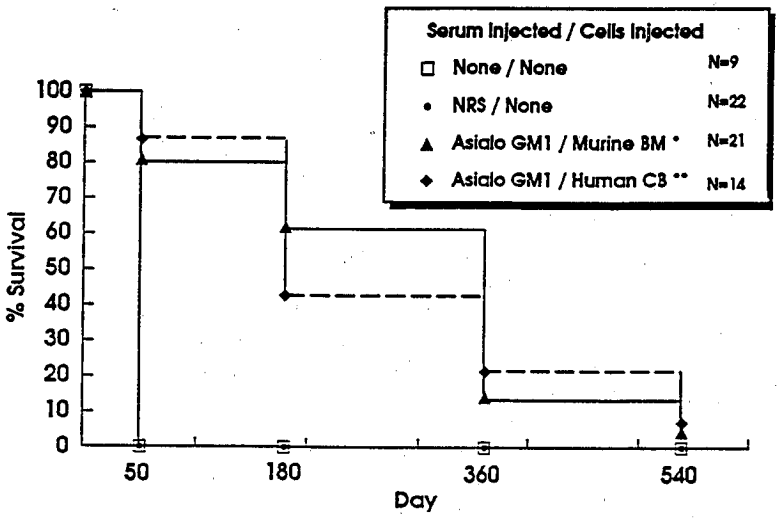
Syngeneic bone marrow cells were obtained by direct, repeated flushing of cells from the tibias and femurs of normal SJL/J donor mice using 2 ml of normal saline delivered from a 3 ml syringe through a 26 ga needle.

Forty ml of human umbilical cord blood was collected directly into 10 ml of Iscoves modification of Dulbecco's medium (Gibco Laboratories, Grand Island, NY) containing 50 units/ml of preservative free heparin. Ficoll hypaque density gradient centrifugation was used to isolate mononuclear cells from the whole cord blood. This was suspended in 200 ul of saline. Bone marrow and cord blood cells were washed 3 times with normal saline and assessed for viability by trypan blue dye exclusion. Cells were given 50% intravenously and 50% of cells intraperitoneally, within 2-12 hours of irradiation. Both routes of administration were utilized because of different techniques described in the literature for the introduction of stem cells.

Injection of 100 ul of anti-Asialo GM_1, antiserum I.V. depletes NK effector cells. This is measured by the inability of splenic effector cells to cause lysis of the NK susceptible target cell, YAC-1, for period of 2 weeks after injection (N.M. Ponzio, unpublished observations). Mice were given 100 ul of anti-Asialo GM_1 antiserum 24 hours before irradiation, and adoptive transfer of hematopoietic cells was done within 2 - 12 hours of irradiation. Mice in control groups received irradiation only or irradiation and 100 ul normal rabbit serum (NRS) in lieu of anti-Asialo GM, antiserum. Rabbit anti-Asialo GM_1 antisera was purchased from Wako Chemicals (Dallas, Texas).

FIGURE 1

Influence of Human Cord Blood on Survival of Lethally Irradiated SJL/J Mice[1]



[1] SJL/J mice were irradiated with 900 cGy. (Sacrificed animals excluded in calculations.)

* These animals received Anti-Asialo GM_1 serum (Natural Killer Cell Antibody) and 4 x 10^6 nucleated bone marrow (BM) cells.

** Animals received Anti-Asialo GM^1 serum and 5 x 10^6 mononuclear cells from human umbilical cord blood (CB).

Human granulocyte macrophage colony stimulating factor (GM-CSF) and Human granulocyte colony stimulating factor (G-CSF) was provided by Genetics Institute, Cambridge, Massachusetts. The animals were injected with GM-CSF subcutaneously three times a week for three weeks. A dose of 700 units of GM-CSF was given per injection. The dose of human G-CSF was 3 units, three times a week for three weeks.

The dosage of cyclosporine (Sandoz Laboratories, East Hanover, NJ) used was 1.25 mg intravenously five times a week for two weeks and was started two days before transplantation.

Results

In one study (Figure 1), where 25 animals (4 were sacrificed) received 900 cGy of irradiation plus anti-Asialo GM_1 serum and HUCB, 13 animals survived for 50 days ($P < 0.05$ by Arova). This was relatively similar to the survival of mice that received irradiation, antibody and syngeneic bone marrow, where 17 out of 25 mice (4 were sacrificed) survived for 50 days. At the end of one year, 3 animals were still alive in each of these two groups. In contrast, the 10 control animals that received only irradiation ,and the 25 animals that received irradiation and normal rabbit sera, were all dead by the 50th day. It is of interest to note that none of the animals received HUCB or syngeneic mouse bone marrow died from lymphoma, which is prone to occur in a high percentage (greater than 80%) of this breed SJL/J when they approach 1 year of age (5,6). Our number of survivors, however, at 12 months are too small to draw conclusions.

In an attempt to improve the survival rate of lethally irradiated mice that were injected with HUCB, agents known to inhibit transplantation rejection or to act as hematopoietic growth factors were also used. In the experimental series shown in Table I, 67% of irradiated mice that received HUCB survived to 130 days, whereas all mice that were irradiated without cellular reconstitution succumbed by day 26. It is important to note that injection of Asialo GM_1 alone afforded no radioprotective effect in the absence of HUCB or syngeneic murine bone marrow.

The survival of mice that received cyclosporine injections in addition to hematopoietic cells was not improved. Indeed, regardless of whether murine bone marrow or HUCB cells were used to reconstitute irradiated mice, the injection of cyclosporine slightly reduced survival measured at 130 days.

Injection of HUCB plus hematopoietic growth factors resulted in a slightly improved survival rate for lethally irradiated mice, not statistically significant. At 130 days, 8 of 9 mice that received GM-CSF and 7 of 10 mice that received GM-CSF and G-CSF, were still alive.

In the series of experiments presented in Fig. I and Table I, a total of 72 mice received lethal irradiation and reconstitution with HUCB (4 were sacrificed). At the end of 50 days, 49 (72%) were still alive. The common denominator in all these studies was that all the animals received human umbilical cord blood and Anti-NK cell antisera. In contrast, there were 55 control animals that received 900 cGy of irradiation without cellular reconstitution, and none of these mice survived to 50 days. These results show unequivocally that xenogeneic hematopoietic cells present in human umbilical cord blood can protect mice against the lethal effects of high dose irradiation.

Discussion

Existing host NK cells are though to be the cell type responsible for bone marrow transplant rejection in both allogeneic and xenogeneic models (7). Since murine NK cells are not destroyed by 900 cGy of irradiation (7), anti-Asialo GM_1 antiserum was used in the present study to remove these cells in vivo. We have previously shown that in the experimental model presented here, injection of Asialo GM_1 antiserum enhances the survival of lethally irradiated mice injected with HUCB. Whether it is advantageous to deplete NK cells in humans is unclear, but it has been suggested that pre-transplant therapy to destroy NK cells be evaluated (8). In our studies and those of others, anti-Asialo GM_1 given without hematopoietic cells does not appear to be "radioprotective" (8). Our results do not totally reveal

the mechanism by which HUCB cells afford protection against irradiation death. It is possible that a temporary functional xenograft has been produced in the animals that survive. This appears to be a likely explanation, since we have been able to identify human DNA by Southern blot and slot blot analysis in such mice up to 6 months after implantation of the HUCB (4). It was postulated that in the Chernobyl radiation disaster, those individual who received a bone marrow transplant obtained a temporary allogeneic graft, which was later replaced by the recipient's own cells (2). This is the mechanism which, in our opinion, best explains the results presented herein. The type and number of hematopoietic cells, as well as their location, is still unclear to us, and it is even possible that the dying HUCB cells may provide (or stimulate) ingredients which allow the animal to survive the critical post irradiation period, until their own hematopoietic system can recover.

TABLE I

INFLUENCE OF CYCLOSPORINE OR REGROWTH FACTORS ON SURVIVAL OF LETHALLY IRRADIATED SJL/J MICE

Cells Injected	Asialo GM_1 Antibody	Cyclosporine	GM-CSF	G-CSF	# of Animals	Survival on Day 26	50	130	270
None	+	-	-	-	10	0	-	-	-
BM*	+	-	-	-	10	5	5	2	2
BM~	-	-	+	+	5	5	5	5	-
CB^	+	-	-	-	9	8	8	6	4
CB^	+	+	-	-	9	7	6	5	4
CB^	+	-	+	-	9	8	8	8	5
CB^	+	+	+	-	10	7	7	6	5
CB»	+	-	+	+	10	7	7	7	-
None	+	-	-	-	10	0	-	-	-

CB - Human cord blood
BM - Mouse bone marrow
GM-CSF - Granulocyte Macrophage Colony Stimulating Factor
G-CSF - Granulocyte Colony Stimulating Factor

* $3.5\ 10^6$ - Nucleated bone marrow cell, SJL/J donor
~ 6.5×10^6 - Nucleated bone marrow cells, SJL/J donor
^ $5.5\ 10^6$ - Mononuclear cells from human cord blood
» 6.6×10^6 - Mononuclear cells from human cord blood

The most significant part of this study, however, is that HUCB cells were able to sustain life in animals receiving lethal irradiation; allowing the animals to survive the critical period post irradiation. Perhaps an extension of these results can be made for therapeutic usage in humans who receive lethal irradiation, where the immunological barriers of transplantation of a xenograft do not exist. There is no indication HUCB would have any different complications than routine blood transfusions (9). There is even the suggestion from the literature that in humans, an exact or close donor-recipient, HLA match is not needed when umbilical cord blood is utilized for transplantation (10). This case report (10) was carried out before HLA typing was readily available and the possibility of a close match at that time (1972) was unlikely. If human umbilical cord blood is as radioprotective in humans as it was in the mice described herein, and a close match may not be necessary, the potential benefit to humans with neoplasms may be of great value.

In conclusion, our results in an experimental model in mice indicate that human umbilical cord blood can protect against irradiation death. Although the exact mechanism by which this protection occurs has not been fully revealed, these results should encourage similar studies in humans. Our results have far-reaching implications regarding, not only the use of HUCB therapy to rescue individuals involved in lethal irradiation accidents, but also in patients who receive whole body irradiation or intensive chemotherapy as part of their treatment for various types of cancer.

Acknowledgement

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