Both Parents as Donors for Bone Marrow Transplantation: Failure to Induce Tolerance and Improve Outcome in Rabbits

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Graft-vs.-host disease (GvHD) is a major limiting factor in clinical histoincompatible bone marrow transplantation (BMT). It can be prevented by the removal of mature T lymphocytes, T-cell depletion. However, the consequences are an increased rate of rejection, long-term immunoincompetence and, in patients transplanted for hematological malignancies, an increased rate of relapse [1-3]. The use of mixed syngeneic and allogeneic T-cell-depleted bone marrow induces specific tolerance and can abrogate these negative effects of T-cell depletion. This approach initially introduced by Ildstad and Sachs prevents GvHD and retains antileukemic efficacy when tested in a leukemia mouse model [4, 5].

A similar concept was proposed by Rammensee [6] in 1986. He postulated that T-cell-depleted hemopoietic cells when transplanted from both parents should mutually tolerate each other in the thymus and generate a mixed population tolerant to all of the offspring's MHC antigens. Simultaneous bone marrow transplantation with T-cell-depleted bone marrow from both parents should therefore lead to full reconstitution and immunocompetence. We have previously attempted to reproduce the results of mixed syngeneic, allogeneic bone marrow transplants in rabbits [7]. We were therefore interested in testing the use of biparental bone marrow transplants in rabbits.

Adult closed colony bred New Zealand white RLA-typed rabbits were used for this experiment (kindly provided by L. Adler, Memphis, Tenn., USA). Parent animals homozygous for RLA class I antigens but with different class I antigens were selected to breed F₁ children defined for their class I antigens (e.g. AA × BB → F₁: AB). This selection enabled us to ascertain that all F₁ recipients were

haploidentical for class I antigens with each of their parents. Four such families were obtained. It was planned to always do a paired transplant, a double transplant from both parents to one F₁ offspring and simultaneously as a control a single parent transplant to one F₁ animal.

Transplant procedure was performed in F₁ animals of at least 1 year of age. The transplant programme was done as previously described [8]. Animals were housed in single cages on pellet diet and water ad libitum. They were conditioned for transplant with 1 mg/kg thiopental i.v. on day -1, and 1,000, respectively, 1,200 cGy total body irradiation (TBI) given at 20 cGy per min, as

previously described [7]. Bone marrow was harvested from the donor animals from both iliac crests under general anesthesia. It was T cell depleted by rosetting at 4°C and incubation with 9 AE10 monoclonal antibody at a concentration of 1:1 in recipient's serum for 1 h to provide complement. The bone marrow transplant was performed 1 day after TBI. All animals were inspected daily, their weight and clinical status was recorded. All were given Bactrim® p.o. during the period of aplasia. Blood samples to measure whole blood counts were taken 3 times weekly, renal and liver function tests were assessed once a week. In long-term survivors, chimerism was assessed by chromosomal analysis, immunoglobulin allotype determination, skin grafting and RLA typing.

Three Fa animals were transplanted with T-cell-depleted bone marrow obtained from both their parents, 2 control animals from a single parent. For the 2 other control animals planned for the experiment not enough cells were obtained. Both control animals died, one on day +1 from TBI, the second on day +17 of GvHD. Two of the 3 study animals given biparental marrow died, one on day

+3 as a result of TBI, one on day +34 of GvHD. One animal became a long-term survivor but showed on examination autologous reconstitution. The total number of nucleated bone marrow cells infused ranged from 1 to 10×10^7 cells (0.3 to 3.4×10^7 /kg). There was no correlation between cell dose and outcome. The animal with the highest cell dose showed autologous reconstitution. The animal given the low dose of 0.3×10^7 /kg died of GvHD.

Rabbits are useful models in bone marrow transplantation. They allow transplantations from an individual donor to an individual recipient while keeping the donor alive [8, 9]. Hence, if successful, tolerance can be tested with skin grafts in an outbred situation. Following a standard conditioning with TBI about 10% of animals die as a result of TBI and about 10% reject or recover autologous reconstitution. This is not ideal. However, conditioning with 900 cGy is followed by autologous reconstitution in 5 of 5 animals, conditioning with 1,200 cGy with TBI mortality in 6 of 10 animals. As shown in previous experiments in different institutions, median survival following mismatched un-manipulated bone marrow transplants in rabbits is 18 days; it is 35 days following T-cell depletion [8, 10, 11].

The number of animals used in these experiments is small and the conclusions are limited. However, the results are compatible with previous findings using T-cell-depleted bone marrow transplants. Median survival of the 3 study animals was 34 days. This corresponds to published results. Death from TBI, stable engraftment as well as rejection or autologous reconstitution are observed in a group of animals all treated identically. The use of bone marrow from both parents at the same time did not alter outcome and failed to provide a benefit. GvHD was observed in 2 animals and no tolerance was induced. It is unlikely that a higher number of transplant experiments would alter this conclusion. The results fit our previous attempts. In an earlier study, we were unable to reproduce the benefit of mixed autologous/allogeneic transplants in rabbits, as has been shown in mice and rats [5]. These failures suggest that the tolerance-inducing approaches used in mice cannot simply be transferred to other animals and to man. It is therefore unlikely that the use of both parents as donors is more beneficial than the use of a single parent transplant, as is current practice for children with immunodeficiencies without HLA-identical siblings [12].

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