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Original Contribution

INDEFINITE SURVIVAL OF RAT ISLET ALLOGRAFTS FOLLOWING INFUSION OF DONOR BONE MARROW WITHOUT CYTOABLATION

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☐ **Abstract — We have tested the effect of donor bone marrow cell (DBMC) infusion on the survival of pancreatic islet allografts in the rat, without the use of cytoablative recipient conditioning. Lewis and diabetic Brown Norway rats were used as donors and recipients, respectively. Donor islets were placed beneath the left renal capsule. Infusion of DBMC and temporary immunosuppression followed by delayed islet transplantation resulted in indefinite survival of all islet grafts (MST >180 days). Control animals demonstrated recurrent hyperglycemia (islet allografts rejection). Donor bone marrow derived cells were detected in the spleen and cervical lymph nodes of BN recipients of LEW bone marrow but not in the recipients of islet transplants alone. Second set full thickness skin grafts were performed in normal BN and in recipients of a previously successful ITX. Donor specific skin grafts were accepted in the animals that had received DBMC 40 days before the islet allograft, while animals receiving DBMC at the time of the islet allograft rejected the donor specific skin graft similarly to the controls. However, these animals did not reject a second set donor-specific islet transplant. The results indicate that radiation conditioning of the recipients was not necessary to induce microchimerism and graft acceptance in this rodent model of islet allotransplantation.**

☐ **Keywords — Tolerance; Islet; Allograft; Bone marrow.**

Indefinite survival of pancreatic islet grafts was recently reported in rodents following radiation conditioning of the recipients and hematopoietic reconstitution with bone marrow cells of donor origin (3,6,8). These models of bone marrow conditioning successfully induced drug-free acceptance of donor specific pancreatic islet allografts. However, the use of lethal or sublethal radiation treatment severely limits the applicability of this approach in humans. Recent experimental evidence indicates that radiation conditioning of the recipient may not be necessary for stem cell engraftment (1,2,7). We have therefore tested the effect of donor bone marrow infusion on the survival of pancreatic islet allografts in the rat, without the use of cytoablative recipient conditioning.

Adult male Lewis (LEW, RT1^l) and Brown Norway (BN, RT1^n) rats were used as donors and recipients, respectively. The recipients were made diabetic by one injection of streptozotocine (65 mg/kg, IV) and only animals with plasma blood glucose levels greater than 350 mg/dL for 2 consecutive days were used as recipients of islet grafts. The islets were obtained by an automated method (4) as previously described (6). Fourteen hundred (1400) highly purified islets of an average diameter of 150 μ were placed beneath the left renal capsule. Donor bone marrow cell (DBMC) infusion consisted of the IV injection of 2.5×10^8 cells either 40 days before or at the time of islet transplantation (ITX). The recipient rats were divided into the following groups. The animals in Group 1 received only the renal subcapsular islet graft without any immunosuppression ($n = 5$). In Group 2, temporary immunosuppression with FK506 (1 mg/kg, IM) on Days −39 to −26, −19, and −12 (Treatment A) was followed by ITX on Day 0 (12 days after discontinuation of immunosuppression) ($n = 4$). In Group 3, temporary immunosuppression with FK506, was administered on days 0 to 13, 21 and 28 days after ITX (Treatment B; $n = 5$). In Group 4, DBMC infusion and ITX were performed simultaneously, in association with the temporary immunosuppression regimen of Treatment B

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(n = 4). In Group 5, DBMC infusion was followed by the temporary immunosuppression regimen (Treatment A). The ITX occurred 12 days after the last FK-506 injection and 40 days following DBMC infusion (n = 5).

In Groups 1 and 2 all animals demonstrated recurrent hyperglycemia (islet allografts rejection) within 2 wk after ITX. There was no significant difference between these two groups. In Group 3, as was predictable, the addition of temporary immunosuppression significantly prolonged the survival of the islet allografts with a mean survival time (MST ± SD) of 76.6 ± 15.0 days. The ITX survival in this group was significantly prolonged compared to Groups 1 and 2 ($p < 0.01$; ANOVA). Nevertheless, all animals eventually demonstrated recurrent hyperglycemia, indicating delayed islet rejection. In Group 4, there was marked prolongation of islet allograft survival to 145.8 ± 8.5 days. ITX survival was significantly prolonged compared to both Groups 1 and 2 ($p < 0.01$) and to Group 3 ($p < 0.05$). Nephrectomy of all kidneys bearing the islet grafts produced a rapid return to the diabetic state, indicating that the transplanted islets were responsible for maintaining normoglycemia. Two animals of this group received a second ITX from the same donor strain in the contralateral kidney (right). These second set grafts were also accepted and normalized plasma glucose levels without evidence of rejection (MST >90 days). In Group 5, infusion of DBMC and temporary immunosuppression (Treatment A) followed by delayed islet transplantation resulted in indefinite survival of all islet grafts (MST >180 days). Histology of the renal subcapsular grafts revealed well-preserved insulin-producing tissue (Fig. 1). Donor bone marrow derived cells were detected in the spleen and cervical lymph nodes of BN recipients of LEW bone marrow using a monoclonal antibody (L-21-6) that reacts with the invariant chain of Class II MHC molecules of LEW and most other rat strains, but not BN (1,5). Donor bone marrow derived cells were identified in the recipients of DBMC, but not in the recipients of islet transplants alone. Nevertheless, donor-derived cells were not detectable by flow cytometric analysis of peripheral blood leukocytes in recipients of DBMC, indicating that microchimerism was present at a level less than 1%.

Second set full thickness skin grafts were performed in normal BN and in recipients of a previously successful ITX. In the latter cases, the skin grafts were performed 4 wk following nephrectomy of the kidneys bearing the functioning islet allografts. The results are summarized in Table 1 and indicate that, as expected, control animals rejected both donor and third party skin grafts. To our surprise, donor-specific skin grafts were accepted only in the animals of Group 5, that had received DBMC 40 days before the islet allograft. The animals in Group 4 rejected the donor-specific skin graft similarly to the controls. However, as indicated earlier, these animals did not reject a second set donor-specific islet transplant. Although it is not possible to draw a firm conclusion from the small number of animals we studied, the results raise the question that donor specific and/or organ specific tolerance can be dependent upon the timing of DBMC infusion.

In summary, the results indicate that radiation conditioning of the recipients was not necessary to induce prolonged microchimerism and graft acceptance in this rodent model of islet allotransplantation, and confirms the results recently reported by Demetris et al. on bone marrow and whole organ allotransplantation (1).

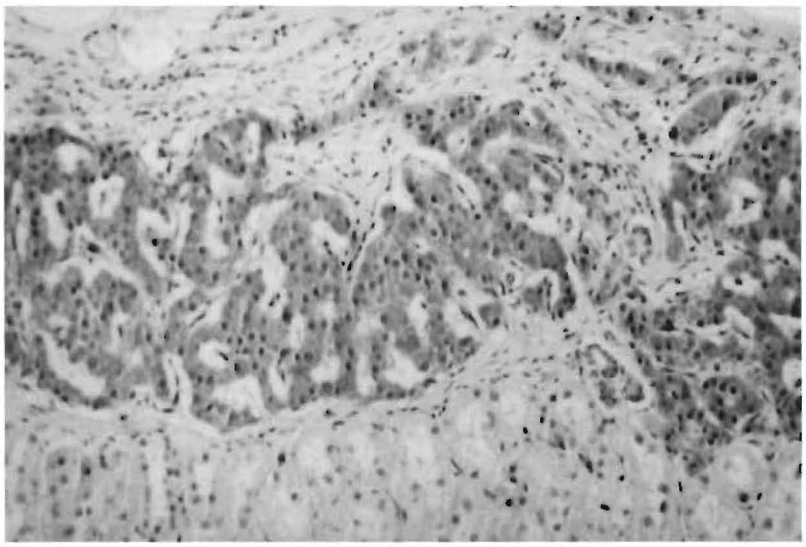

Fig. 1. Renal subcapsular islet allograft (dark) stained for insulin (immunoperoxidase) 220 days following transplantation. Original magnification ×200.

Table 1. Skin graft survival

Recipients	Skin Graft Survival (Days)		
	ACI skin	LEW skin	BN skin
Normal BN (Controls)	10, 10	10, 11	>70, >70
Group 4 (ITX + DBMC + Treatment A)	10, 12	11, 12	>70, >70
Group 5 (DBMC + Treatment B → ITX)	12, 12	>70, >70	>70, >70

The paradigm of "making space" by cytoablative conditioning has been recently challenged, indicating that it is possible to infuse a high dose of donor marrow to successfully and permanently repopulate a recipient without radiation treatment (2,7). These results may be even more relevant when the target of DBMC treatment is the generation of a microchimeric state. Our results may be of assistance in defining new strategies for transplantation of donor hematopoietic cells to enhance allograft survival.

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