Rapid Publication

Prolongation of Islet Xenograft Survival by Cryopreservation

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SUMMARY

Our attempt to reduce islet immunogenicity by slow cooling to −40°C, storage at −196°C, and rapid thawing is based on the differential susceptibility of various cell types to a freeze-thaw process. Five hundred rat islets (≥100 μm) were immediately implanted or cryopreserved and then implanted beneath the renal capsule of streptozocin-induced diabetic mice with or without an injection of anti-lymphocyte serum at the time of transplantation. Thirteen days after transplantation, all fresh xenografts had rejected, whereas 37.5% of cryopreserved grafts were still functioning. In immunosuppressed mice, 6.2% of fresh xenografts and 54.5% of cryopreserved grafts were functioning 19 days after transplantation. These results show that cryopreservation can extend xenograft survival. *Diabetes* 36:1086–88, 1987

Pretreatment regimens such as in vitro culture (1,2), anti-Ia serum plus complement (3), or ultraviolet irradiation (4) have successfully prevented the rejection of rodent pancreatic islet allografts. The survival of concordant (rat to mouse) islet xenografts has been markedly prolonged by low-temperature culture (24°C) in conjunction with short-term anti-lymphocyte serum (ALS) (5) or cyclosporin (6), or 37°C culture of rat megaislets in 95% O_2 (7). These procedures are aimed at the selective elimination of immunostimulatory antigen-presenting cells, an approach based on the concept that passenger leukocytes residing in donor tissue provide the major barrier to successful tissue transplantation (8).

Cryopreservation of isolated islets or pancreatic fragments has yielded viable tissue that can ameliorate experimentally induced diabetes in rodents and larger animals (9–11). Because distinct cell types are known to be differentially susceptible to a particular freeze-thaw process (12), it is conceivable that islet immunogenicity may be modulated with a cryobiological approach. We examined this possibility in a xenograft model by a cryopreservation protocol known to yield islets that are functional in vivo.

MATERIALS AND METHODS

Animals. Male Wistar-Furth rats (Harlan-Sprague-Dawley) were used as islet donors. Recipients were male BALB/c mice obtained locally. The mice were individually housed in metabolic cages, and baseline nonfasting plasma glucose (PG), urine volume, urine glucose, and weight were monitored. Mice were anesthetized with tribromoethanol (Aldrich, Milwaukee, WI) dissolved in tertiary amyl alcohol (Fisher, Silver Spring, MD) (0.01 ml/g) (13) and were rendered diabetic with streptozocin 200 mg/kg body wt via the retroorbital sinus. Clinical indices were monitored three times over 2 wk before transplantation.

Isolation and cryopreservation of islet tissue. Islets were isolated by collagenase digestion and Ficoll purification and were meticulously handpicked free of exocrine contamination as previously described (9). Aliquots containing 500 islets ≥100 μm were cryopreserved or immediately transplanted.

The freeze-thaw sequence used was that of Rajotte et al. (10). Briefly, dimethyl sulfoxide (DMSO) was added stepwise to a final concentration of 2 M. The samples were supercooled to −7.3°C, nucleated, slowly cooled at 0.25°C/min to −40°C, then quickly frozen to −196°C for storage. The samples were rapidly thawed (200°C/min) in a 37°C water bath, and the hyperosmolar DMSO was removed with 0.75 M sucrose at 0°C. The sucrose was diluted with stepwise additions of isotonic media at 25°C.

Transplantation. In all cases, 500 islets were embedded in a blood clot and inserted beneath the kidney capsule of recipient mice having PG values >500 mg/dl. Mice in group 1 and group 2 received freshly isolated or frozen-thawed

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TABLE 1
Effect of cryopreservation and anti-lymphocyte serum (ALS) on islet xenograft survival (rat to mouse)

Group	Treatment (τ*)	*n*	Day of rejection†
1	Fresh (control)	11	10, 10, 11, 11, 11, 11, 12, 12, 13, 13, 13
2	Cryopreservation	8	8, 10, 11, 11, 13, 14, 16, 17
3	Fresh + ALS‡	16	13, 14, 15, 15, 15, 16, 16, 17, 17, 18, 18, 19, 19, 19, 19, 26
4	Cryopreservation + ALS‡	11	16, 16, 16, 17, 18, 20, 23, 23, 28, 29, 37

*Jonckheere-Terpstra test for ordered alternatives: $J^* = 5.576 > z(0.001) = 3.090$. $\tau_1 < \tau_2 < \tau_3 < \tau_4$ is significant at the 0.1% level.
†Defined as the first of 3 consecutive days of plasma glucose ≥200 mg/dl.
‡Mice received 1 injection of rabbit anti-mouse and rabbit anti-rat lymphocyte serum at time of transplantation.

islets, respectively. Mice in groups 3 and 4 received fresh or cryopreserved islets, respectively, in conjunction with a single injection of rabbit anti-mouse lymphocyte serum (0.2 ml) and rabbit anti-rat lymphocyte serum (0.1 ml) administered intravenously 5 min before transplantation. All clinical indices were monitored daily until rejection (defined as the first of 3 consecutive days of plasma glucose ≥200 mg/dl) was evident.

RESULTS

In each group, all clinical indices returned to baseline values within 2–4 days after islet transplantation. Once graft rejection was initiated, it was extremely rapid, reaching pretransplant diabetic values within 5 days. As shown in Table 1, freshly isolated rat islets implanted beneath the kidney capsule of diabetic mice (group 1) were rejected in 10–13 days, and cryopreserved islets (group 2) were rejected in 8–17 days. When ALS was administered, graft survival was extended to 13–26 days in the fresh and 16–37 days in the cryopreserved groups. Analysis by the Jonckheere-Terpstra test for ordered alternatives (14) showed that treatment groups 1–4 had an increasing effect on extending xenograft survival, and this was highly significant. The effect is demonstrated in Fig. 1. Beyond 10 days after transplantation, the groups of mice that received cryopreserved islets, with or without ALS, showed greater proportions of surviving grafts than their fresh counterparts. Twelve days after transplantation, 27.3% of fresh islet xenografts and 50.0% of cryopreserved grafts were surviving. After 13 days, all fresh xenografts had rejected, but 37.5% of cryopreserved grafts were still functioning. When ALS was given, 54.5% of cryopreserved grafts and 6.2% of fresh grafts were functioning 19 days after transplantation. After 26 days, when all fresh grafts had rejected, 27.3% of frozen-thawed grafts were still able to normalize the animals.

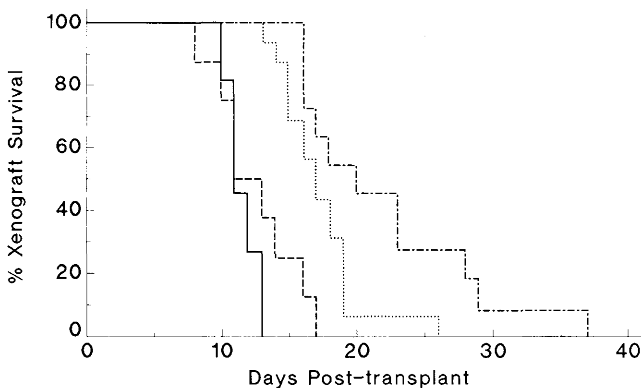

FIG. 1. Percent xenograft survival of fresh (*solid line*) or cryopreserved (*dashed line*) islets and of fresh (*dotted line*) or cryopreserved (*dotted-and-dashed line*) islets in conjunction with a single injection of anti-lymphocyte serum at time of transplantation.

DISCUSSION

Cooling rates optimal for cell survival can vary widely for different cell types (12). Thus, a given cryopreservation protocol can selectively destroy cells of one type and preserve the function of another type. Cryopreservation of canine pancreatic fragments can simultaneously destroy exocrine tissue and preserve endocrine function (9,15). This differential susceptibility of various cell types to freeze-thaw damage was tested on isolated rat islets in an attempt to selectively destroy or inactivate immunocompetent passenger leukocytes. Our results indicate that cryopreservation can reduce islet immunogenicity. The survival of 500 fresh rat islets inserted beneath the kidney capsule of nonimmunosuppressed mice compares with that reported by Bobzien et al. (16). A greater percentage of cryopreserved islet xenografts, with or without ALS, showed extended graft survival; however, survival was only moderately prolonged compared with cultured islets (5–7) or cryopreserved islet allografts (17). Although this extended xenograft survival may be a result of exocrine tissue destruction, it appears that the cryopreservation protocol does eliminate or inactivate passenger leukocytes from the islet graft, because the islets were carefully handpicked free of exocrine tissue. Some passenger leukocytes, however, may have survived. Taylor et al. (18) have shown that lymphocytes and macrophages are more susceptible to damage at fast cooling rates (75°C/min). Further investigations should include detection of Ia-positive cells within the islets before and after freezing and examinations of other freezing protocols that will achieve maximum destruction of immunostimulatory antigen-presenting cells without affecting endocrine function. The ability of cryopreservation to reduce islet immunogenicity and establish a low-temperature tissue bank could have important applications to clinical islet cell transplantation.

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