Rapid Publication

Encapsulation of Rat Islets of Langerhans Prolongs Xenograft Survival in Diabetic Mice

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SUMMARY

Rat islets encapsulated in alginate-polylysine membranes were implanted intraperitoneally into nonimmunosuppressed streptozocin-induced diabetic mice. Diabetes was reversed within 3 days, and the animals remained normoglycemic for up to 144 days, with a mean xenograft survival of 80 days. This was significantly greater than nonencapsulated islets, which functioned for <14 days. The graft survival rate at 50 days was >80%. Xenografts of rat islets encapsulated in alginate-polyornithine membranes also had a prolonged survival rate. This study demonstrates that encapsulation of pancreatic islets in semipermeable membranes can prolong xenograft survival in the absence of immunosuppression. DIABETES 1986; 35:943-46.

The problems of immune rejection continue to be the major obstacle to islet transplantation as a treatment for type I diabetes. A possible solution is to protect the transplanted islets from the host's immune system by means of semipermeable membranes. The two principal techniques currently under investigation are 1) the use of hollow-fiber artificial endocrine pancreas (AEP) devices1-5 or 2) microencapsulation of the islets.6-9 Coagulation problems, either within the devices themselves or at the site of vascular connections, have limited the functional life span of AEPs. Studies with the microencapsulation technique have yielded more promising results. Previous reports from our laboratory demonstrated that intraperitoneal islet allografts can be protected from rejection for up to 21 mo in non-immunosuppressed, streptozocin (STZ)-induced diabetic rats by encapsulation in alginate-polylysine-alginate membranes.8,9 Furthermore, when the initial grafts failed, a second transplant of encapsulated islets again successfully reversed diabetes with prolonged graft survival.8 Our study was undertaken to determine if the capsule membrane would also prolong xenograft survival in the absence of immunosuppression.

MATERIALS AND METHODS

Animals. Outbred male Wistar rats (Charles River Canada), weighing 250–300 g, were used as islet donors, and male BALB/c mice, 18–20 g, were transplant recipients. The mice were made diabetic by injection of STZ (Sigma, St. Louis, MO) at a dosage of 220 mg/kg i.v. Blood samples for determination of fasting plasma glucose concentration were obtained weekly by bleeding from the orbital sinus. Only mice with persistent fasting plasma glucose concentrations >360 mg/dl were used as transplant recipients. Failure of transplanted islets was considered to have occurred when the first of two consecutive glucose concentrations >200 mg/dl was obtained. Plasma glucose was determined with Beckman glucose analyzer 2.

Islet isolation and culture. Islets were isolated from rat pancreas by a standard collagenase (type V, Sigma) digestion technique10,11 and handpicked with the aid of a dissecting microscope. The isolated islets were cultured before encapsulation for 1–3 days at 37°C in CMRL-1969 supplemented with 7.5% (vol/vol) fetal bovine serum, 300 mg/ml glucose, and 50 μg/ml gentamicin.

Islet encapsulation. The encapsulation method with polylysine was similar to that described previously.9,9 Islets were suspended in 1.3% sodium alginate (KelcoGel LV, Kelco, NJ) at a concentration of 2–3 × 10^6/ml; droplets of this suspension were formed by syringe-pump extrusion and gelled on collection in a 1.1% CaCl_2 solution. The gels were washed with 25-ml volumes of 1) 0.55% CaCl_2, 2) 0.27% CaCl_2, 3) 0.85% NaCl, and 4) 0.1% 2-N-(cyclohexylamino)ethanesulfonic acid (CHES), pH 8.2, and suspended in 0.05% poly-L-lysine (Sigma; M, 16,000–22,000) for 10 min. Sequential washes with 1) 0.1% CHES and 2) NaCl were followed by suspension in 0.15% sodium alginate for 4 min. After washing again with NaCl, the alginate within the capsules was reliquified by suspension in 10 ml of a 55-mM sodium citrate solution for 6 min. The capsules were finally washed once...
with saline, twice with culture medium, and then incubated at 37°C. Each capsule contained one to two islets and had a diameter of 0.7–0.8 mm.

To encapsulate islets in alginate-polyornithine membranes, the initial entrapment and gelation steps were performed as described above. The gel droplets were washed three times with 0.85% NaCl and then with 0.2% piperazine-A/-N'-bis(2-ethanesulfonic acid) (1,4-piperazinediethanesulfonic acid; PIPES) and suspended in 0.12% poly-L-ornithine for 10 min. After washing with 0.85% NaCl, the beads were suspended in 0.05% sodium alginate for 4 min, again washed with saline, and the alginate reliquified by suspension in 55 mM sodium citrate for 10 min; the sodium citrate treatment was repeated for 6 min. The capsules were finally washed twice with saline and culture medium and incubated at 37°C.

**Islet xenografts.** Under light ether anesthesia, diabetic mice received intraperitoneal transplants of 1000 encapsulated islets, nonencapsulated islets, or empty capsules suspended in 2 ml of 0.85% NaCl. The implants were made through a small incision with a 14-gauge cannula attached to a 5-ml syringe.

**RESULTS**

Twenty-two STZ-induced diabetic BALB/c mice received intraperitoneal transplants of 1000 rat islets encapsulated in alginate-polylysine membranes. In all cases, diabetes was reversed within 3 days; fasting plasma glucose levels dropped from a pretransplant mean of 485.8 (SE ± 13.7) mg/dl to 95.8 ± 8.7 mg/dl. The xenografts functioned for periods ranging from 28 to 144 days with a mean survival time of 80.0 ± 6.6 days (Table 1). Eighteen of the 22 grafts functioned for >50 days. Thirteen animals received a second xenograft of 1000 encapsulated islets 2–3 wk after the reappearance of hyperglycemia. The recipients again became normoglycemic and remained so for periods ranging from 16 to 110 days posttransplantation. Diabetes was also reversed in recipients of 1000 nonencapsulated rat islets, but these unprotected xenografts functioned for <14 days.

Intraperitoneal implants of empty capsules had no effect on plasma glucose concentrations in diabetic mice (Table 1). Recipients of empty capsules and untreated diabetic animals remained hyperglycemic and had a high mortality rate within 3 mo of diabetes induction.

Ten diabetic mice received intraperitoneal transplants of 1000 rat islets encapsulated in alginate-polyornithine membranes. In all cases, diabetes was reversed within 3 days, and normoglycemia persisted for up to 93 days with a mean duration of 38.6 ± 8.1 days (Table 2). In the eight animals that received a second transplant of alginate-polyornithine–encapsulated islets, diabetes was again reversed, and the functional life span of these xenografts was similar to the initial grafts (Table 2).

Polylysine capsules were recovered from 12 transplant recipients. The polylysine capsules were generally found freeflowing throughout the abdominal cavity, but some clumping of the capsules was observed. The capsule surfaces had varying degrees of cell overgrowth, ranging from 0 to 10 cell layers in thickness. The cells included fibroblasts, macrophage-like cells, and neutrophils. Collagen was also present around the capsules. Islets were found in many cross sections of the recovered capsules. Some islets contained wellgranulated β-cells as shown by aldehyde thionine staining (Fig. 1A). Atrophic cells (Fig. 1B) and cell debris were seen in some capsules. Few of the capsules were broken.

The polyornithine capsules recovered from eight recipients were generally clumped and were more severely overgrown than the polylysine capsules; the cell composition surrounding the capsules was similar. No viable islets were found.

**DISCUSSION**

It has been reported that encapsulation of islets in membranes composed of polylysine and alginate can prolong allograft survival in nonimmunosuppressed STZ-induced diabetes. The present study confirms this finding and extends it to xenografts of 1000 encapsulated islets.

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**Mean** 38.6 ± 8.1  33.8 ± 6.4
FIG. 1. Alginate-polylysine capsules recovered from diabetic mice with granulated β-cells (A) and atrophic islet cells (B). Sections were stained with aldehyde thionine. Magnification × 250.

Abetic rats. Single intraperitoneal allografts of $5 \times 10^3$ encapsulated islets reversed diabetes in rats for up to 21 mo. The permeability of the capsules to glucose, insulin, arginine, and theophylline was also reported. The results described here clearly demonstrate that the capsule membrane can also prolong xenograft survival in nonimmunosuppressed animals. In all of the animals transplanted with 1000 rat islets encapsulated in alginate-polylysine membranes, diabetes was reversed, and $>80\%$ of the xenografts functioned for $>50$ days. A second xenograft of 1000 encapsulated islets, given after the failure of the initial transplant, also had a prolonged survival rate. We had previously observed this same prolongation of second allograft transplants in rats. Xenogenic islets encapsulated in alginate-polyornithine membranes also had a prolonged survival rate that could be repeated with a second transplant. The reason for the difference in islet-graft survival rates, observed with the two different polyamino acids, is unknown. It may be related to differences in the porosity or other physical properties of the membranes. It is suspected that the failure of the grafts can be attributed to the gradual death of the islet cells resulting from lack of nutrients as the capsules became overgrown.

With evidence accumulating that type I diabetes is an autoimmune disease, the possibility of the disease recurring in the transplanted islets is also a concern. It has been documented that the autoimmune disease glomerulonephritis has a high incidence of recurrence in kidney transplants, and autoimmune chronic active hepatitis has also recurred in a liver transplant. More significantly, Sutherland et al. reported the failure of technically successful segmental pan-
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cretic grafts between identical twins where the possibility of rejection can be excluded. On the evidence of biopsy samples, these graft failures appeared to be due to a recurrence of the disease, because mononuclear lymphocyte infiltration of the islets (insulitis) was observed in the grafts. Although altering the immunogenicity of islets before transplantation with various in vitro culture techniques has been shown to prolong both allograft and xenograft survival, these grafts would remain unprotected from destruction by a recurrence of the autoimmune disease. Darquy and Peach have recently shown that the alginate-polylysine membrane protects rat islets and RIN insulinoma cells in vitro from cytotoxic anti-islet antibodies present in serum from rabbits immunized with rat islet cells and newly diagnosed type I diabetics. The capsule may therefore protect transplanted islets both from graft rejection and from autoimmune destruction, although some modification to the membrane may be necessary to improve the biocompatibility of the capsules and to prevent cell overgrowth.

In summary, we have shown that encapsulation of islets in alginate-polyamino acid membranes prolongs xenograft survival in diabetic mice without the need for immunosuppressive therapy. In view of the small number of human pancreas donations annually, it may be necessary to use xenogenic islet sources for human transplants if the islets can be protected from rejection. Our results indicate that encapsulation in semipermeable membranes can provide this protection.

ACKNOWLEDGMENTS

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REFERENCES


